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PRINCIPAL INVESTIGATOR: **Andrew D Shaw, MD**

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14. ABSTRACT
This project continues to be a biomarker discovery program focusing on the causes of persistent pain after traumatic amputation in the combat setting. In the second year we have: 1) maintained the necessary regulatory approval at WRMC and Duke University. We have obtained and maintained documents for approval by MRMC; 2) maintained our interactive, secure web based data collection system; 3) populated our biorepository at Duke with bioresource collected from 71 patients enrolled at WRNMMC; 4) conducted further on-site visits and investigator meetings at WRNMMC; 5) submitted samples to the Duke Core Lab facilities for proteomic, metabolomic, genomic, genetic and epigenetic assays. We have received our initial pilot results and presented these in Washington DC at ASA. We have published two more papers and are now collecting our validation cohort of patients. Our overall progress is slightly ahead of target, given the lengthy administrative delays during year one. Lastly, we have been funded by DOD to conduct an intervention trial at WRNMMC and the Durham VAMC of valproate for the prevention of amputation induced chronic pain.

15. SUBJECT TERMS
Biomarker discovery, traumatic amputation, phantom limb pain, neuropathic residual limb pain, post-amputation limb pain

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INTRODUCTION

The primary purpose of this prospective cohort study project is to identify circulating biomarkers of persistent post-amputation pain among patients who develop persistent (greater than 3 months) phantom limb pain or neuropathic residual limb pain within three months (and less than 18 months) of their amputation as compared to those patients who do not. Patients with persistent post amputation limb pain are being assigned to the case cohort as determined by an expert panel within the research team utilizing the various pain scales (LANSS) or pain type questionnaires (PLP, RLP, CRPS).

The project aims include develop a proteomic model of neuropathic pain, such that the model is able to correctly assign class (i.e. case or control) in more than 95% of cases, and interrogation of the components of the models (using mass spectrometry [MS] peptide identification technology) to reveal those proteins which represent circulating qualitative and quantitative biomarkers of pain.

The primary outcome variable is "the cumulative incidence of amputation pain in the first twelve months after injury related limb amputation." The project is divided into four tasks – human subject approval and enrollment, biomarker discovery, genotyping and re-sequencing for rare variant discovery. All tasks are on target at the time of this second annual report. We anticipate the possibility that we may be requesting a no-cost extension to this project in order to allow time for completion of patient enrollment and data analysis (there was a significant delay to commencement of enrollment because of regulatory delays at WRNMMC in the first year).

BODY

Overall progress is reported according to the tasks laid out in the SOW. The SOW text is italicized, with the summary annual report text immediately following. Reports from each quarter are included after the summary to provide detail on our progress through the year.

STUDY TASK 1

We will enroll subjects between 3 and 18 months after amputation for traumatic injury in an observational study of different subtypes of post amputation chronic pain.

(a) Human subjects approval.

We expect this subtask to take 6-9 months. We will obtain IRB approval at Walter Reed Army Medical Center, in conjunction with our collaborator Dr Buckenmaier and his colleagues at DVPMI. We will submit the initial request for approval by the end of January 2011, and are advised the process is lengthy and may require several resubmissions. The first project milestone is thus IRB approval to enroll subjects at WRAMC, and we expect to reach this no later than 10/1/2011.

(b) Human subject enrollment.

We expect to enroll 165 amputee soldiers at WRAMC over the course of this 3-year project. The second project milestone is enrollment of the first subject by 12/1/2011. In order for the proteomic experiments to have sufficient power we need a minimum of 90 subjects. We will try and enroll as many subjects as we can in the first 12 months after IRB approval. There are several hundred potentially eligible patients undergoing treatment at MATC as of December 2010. The third project milestone is thus enrollment of a minimum of 90 subjects by 12/1/2012.

STUDY TASK 1 – YEAR 2 SUMMARY REPORT

In addition to maintaining ongoing regulatory approval for this project, we have continued to enroll patients and, in fact, have caught up somewhat since our slow first year. We expanded the inclusion criteria to now allow multiple amputees into the study and, at time of this submission, there are 71 patients enrolled in the project. This has allowed us to lock an initial discovery cohort of 60 patients as of December, 2012. These patients will represent a cohort of biomarker discovery patients and we can now proceed with metabolomic, proteomic, genomic and epigenomic discovery experiments. We are extremely pleased to report near 100% completion of the case report forms, with less than 1% missing data. A final year adjudication meeting was held on January 3rd at which the VIPER60 discovery cohort were formally adjudicated (see meeting minutes). In brief, there are 60 patients of whom 38 (63%) are cases and 22 (37%) controls. The incidence of residual limb pain is 34/60 (57%) and of phantom pain 35/60 (58%). The subtypes of residual limb pain are neuroma (22/60), mosaic (3/60), CRPS (8/60) and somatic (11/60). These incidences are entirely in keeping with the previously published rates and mean our biomarker discovery program can now commence (see below).

STUDY TASK 2 – BIOMARKER DISCOVERY (AIMS 1 & 2)

i. Proteomics

Duration	*3 months*	
Milestone	*First data back from Duke Core*	*2/28/12*

ii. Genotyping

Duration	*3 months*	
Milestone	*First data back from Duke Core*	*5/30/12*

iii. Sequencing

Duration	*3 months*	
Milestone	*First data back from Duke Core*	*8/31/12*

STUDY TASK 2 – YEAR 2 SUMMARY REPORT

Proteomic and Metabolomic Discovery Subtask (i)

We have made good progress with biomarker discovery this year. We sent an initial sample of 15 subjects to Metabolon Inc for metabolomics assays in the second quarter. From this we learned that our sample and data handling pipeline worked exactly as we planned – all samples were tracked in and out of our LIMS with no loss of bioresource. We received data back in good time from Metabolon and spent several weeks analyzing their results. These were presented as a series of 5 poster presentations at the American Society of Anesthesiologists Annual Meeting in Washington DC, October 2012. These 15 samples are now being supplemented by full metabolomics analyses on the remaining 45 discovery cohort subjects. Samples have been sent to Metabolon, and data are expected back in Q1 2013.

Paralleling our human biomarker and novel pathway discovery work in humans is a mouse peripheral nerve injury model that has been developed under the direction of another member of our lab, Dr. Thomas Van de Ven. This project utilizes funding from an NIH T32 training grant awarded to the Duke Department of Anesthesiology to produce a mouse peripheral nerve injury model that approximates the pathology present in human amputees. We have performed metabolomic and proteomic analysis of various mouse tissues from this model, including blood plasma, for cross-species verification of potential biomarkers of interest. We believe that metabolic and proteomic signatures that are evolutionarily conserved are more likely to be significant as biomarkers and signposts toward novel pain pathways.

Genotyping and Epigenetic Discovery Subtask (ii)

The field has progressed since we first submitted the application for funding for this project, such that we are now in a position to be able to afford to sequence the entire exomes of all subjects recruited into VIPER. This presents an enormous opportunity for rare variant discovery, and we have submitted a pilot series of 4 samples to the Duke Sequencing Core in order to test the data-flow pipeline. We anticipate following up with the remaining 54 samples once the average coverage data are returned from the cire facility. We anticipate this occurring in February, at which time we will submit the remainder of our discovery samples. These experiments will take 12 weeks, after which the data analysis will take several months. Therefore, we anticipate meaningful results in Q3 of 2013.

In Q4 2012, we have simultaneously submitted samples to the Epigenetic Core facility, the microRNA discovery facility and the gene expression core facility. This means that we are now in a position to analyze the genome, epigenome, microRNA expression and gene expression signatures of 60 prospectively collected samples, with a case rate of 57%. This has never been done before, and represents an incredible opportunity to learn the mechanisms underlying the transition from acute to chronic pain in military amputees. We are very excited that we are now progressing into the data analysis phase of this project, and look forward to sharing details in future reports, as well as disseminating these findings in the medical literature.

Study Task 3 – Biomarker Validation (Aims 1 and 2)

i. Proteomics

Duration	*3 months*	
Milestone	*First data back from Duke Core*	*8/31/12*

ii. Genotyping

Duration	*3 months*	
Milestone	*First data back from Duke Core*	*11/30/12*

iii. Sequencing

Duration	*3 months*	
Milestone	*First data back from Duke Core*	*2/28/13*

Year 2 Quarterly Report Summaries

Quarter 1

- Frozen blood samples for the first 16 patients have been received at Duke and entered into an LMS tracking system.
- Data for the first 16 patients has been reviewed by Drs. Shaw, MacLeod and Buchheit.
- The phenotype adjudication process has been completed and the committee met, adjudicating pain phenotypes of the first 16 patients in the study. Discrimination was determined between phantom pain, residual limb pain-neuroma, residual limb pain-CRPS and residual limb pain-mosaic neuralgia.
- Lab Supplies/Sample Kits – A total of 48 blood sample kits have been sent to Dr. Buckenmaier's research team at Defense & Veterans Center for Integrative Pain Medicine (DVCIPM) for sample collection.

- Re-Budget – A request for a re-budgeting of funds was initially emailed to Ms. Margaret Lesnow on August 4, 2011. Our project coordinator was in touch with Ms. Lesnow on December 21. We were asked to provide a breakdown of costs for the yearly investigators conference in the budget. This was addressed and a revised re-budget request was emailed to Ms. Lesnow on February 7, 2012. We are awaiting word from Ms. Lesnow for this re-budget request.

Quarter 2

- Frozen blood samples for a total of 30 patients have been received to date at Duke and entered into a LIMS tracking system.

- Data for these 30 patients has been reviewed by Drs. Shaw, MacLeod, Buchheit and Van de Ven.

- An initial phenotype adjudication process was completed during the first quarter of year 2 for 16 patients. An official adjudication meeting took place on May 15 when the first 30 patient records were discussed and reviewed.

- Lab Supplies/Sample Kits – To date, a total of 48 blood sample kits have been sent to Dr. Buckenmaier's research team at Defense & Veterans Center for Integrative Pain Medicine (DVCIPM) for sample collection.

- Re-Budget – On June 6, Ms. Lesnow asked for clarification re: funds requested for publication costs in the re-budget. Clarification was sent back to her same day. As of June 6, the re-budget request is still pending approval.

- Yearly Investigators Meeting – Drs. Shaw, Buchheit and Mary Kirkley traveled to Bethesda to meet with the DVCIPM group on Wednesday, May 30, 2012. We met with Col. Buckenmaier and his research team involved in the VIPER project. We discussed patient enrollment and sample collection processes, use of the thermal camera for maximum benefit and first findings from adjudication.

  Mary McDuffie, Research Nurse who screens, approaches, consents and collects blood and questionnaires from study subjects at WRNMMC arranged a lunch time seminar where Dr. Shaw delivered a presentation to PT staff, administrators and amputees who elected to attend. Both DVCIPM and Duke research teams were present. In layman's terms, Dr. Shaw summarized the goals of the VIPER project and the information was very well-received.
- VIPER Website – A website is under construction for the Viper project and should be current within the next 3 months.
- Samples for biomarker discovery, including metabolomics, proteomics and gene expression have been processed and an initial pilot cohort of 15 patients sent out for measurement. These data are expected back in 6-8 weeks.

Quarter 3

- An IRB Amendment was submitted to the Dept. of Clinical Investigation at WRNMMC in mid-July, 2012 requesting approval to recruit service members who have amputations of up to three limbs and possessing at least one intact upper limb to increase eligibility.

- Frozen blood samples for a total of 41 patients have been received to date at Duke and entered into our LIMS tracking system, FreezerPro.

- Data for these 41 patients has been reviewed by Drs. Shaw, Buchheit and Van de Ven.
- Lab Supplies/Sample Kits – To date, a total of 56 blood sample kits have been sent to Col. Buckenmaier's research team at Defense & Veterans Center for Integrative Pain Medicine (DVCIPM) for sample collection.

- Re-Budget – Our re-budget request was approved on June 26.

- Another adjudication meeting was held last week on September 12. Dr. Shaw, PI has prepared a 17-page Pilot Data report which gives details regarding findings from the meeting and analyses which has taken place to date.

- *Yearly VIPER Investigators Meeting Notes authored by Andrew Shaw, PI on May 30, 2012*:
  1. A visit was made on 5/30/2012 to the WRNMMC VIPER site for the purpose of reviewing progress so far, discussing issues arising from the first cohort of patients, and to make plans for follow-on funding applications.
  2. PASTOR. There is much interest in the use of PASTOR in ongoing pain projects, and future funding applications should include this data collection tool. Nancy Kwon will send details of the collaborators at Northwestern University for Dr Shaw to contact for ongoing DOD funding applications.
  3. The DOD pain task force document was discussed, and general agreement that future collaborations should be based around the areas and missions identified by this important document. Col Buckenmaier pointed out the relevance of maintaining close proximity to this military medical mission.
  4. Slides of Dr Shaw's talk were requested by Nancy Kwon and Mary McDuffie. These will be sent on return to Duke.
  5. Amendment to protocol. It was felt that the Von Frey filament testing and mechanical threshold testing was not informative, and that their removal could reduce the testing time without loss to the study. There was agreement to include this in the upcoming protocol amendment. There was discussion about expanding eligibility to bilateral/multiple amputees, since these patients now represent a significant portion of the amputee population at WRNMMC. It was felt that the VIPER mission should reflect the ongoing patient population and therefore that eligibility should be expanded such that these patients have an opportunity to take part. Agreement was reached to include this in the amendment. Heterotopic ossification is also important for rehabilitation, and thus it was agreed to start collecting this datapoint also. WRNMMC staff will prepare a draft amendment and circulate prior to 6/13/2012 for review prior to submission.
  6. Investigators will meet during the American Society of Anesthesiologists (ASA) Conference in Washington DC in October 2012. Meeting summary to follow in our year-end summary.
- Analyses - Samples for biomarker discovery, including metabolomics, proteomics and gene expression were processed and an initial pilot cohort of 15 patients was sent out for measurement at the time of our last technical summary submission. These data are now received back from the lab and we are analyzing them. Initial review shows many promising features, with several known and 4 unknown (ie novel) biomarkers of persistent pain identified. These data will be further

reviewed and then placed in the context of the accompanying gene expression and proteomic experiments now being planned (full report attached).

Quarter 4

· An additional 30 patients were enrolled for a total now of 71 patients. The first 60 of these represent the VIPER discovery cohort and the remaining represent the first 11 in the validation cohort.

· IRB approval was received on October 9, 2012 from WRNMMC for an amendment requesting eligibility of service members who have amputations of up to three limbs with at least one intact upper limb as well as some other minor changes.

· Samples were sent for epigenetic, microRNA, gene expression, sequencing and metabolomics experiments.

· An adjudication meeting was held on January 3, 2013, meeting notes are below.

VIPER Investigators' and Adjudication Meeting Notes

Andrew Shaw, MD

1/04/2013

1. Investigators met during the American Society of Anesthesiologists (ASA) Conference in Washington DC in October 2012. It was agreed to enroll multiple amputees, but to collect data regarding the most painful side in case of double amputation.
2. There was much discussion of the group's second project (valproate study, funded this year with Dr T Buchheit as PI). It was agreed that the new study would not impair enrollment into VIPER, and that every opportunity should be sought for continuation funding for VIPER given how successful the project is currently. All parties will continue to track DOD funding announcements for suitable mechanisms that may be relevant.
3. 30 patients were adjudicated on 1/3/2013. Those present were Dr Andrew Shaw (no vote), Dr Thomas Buchheit, Dr Thomas Van de Ven, Dr David Macleod and a new team member, Dr Matt Mauck, who is a resident physician interested in pain medicine.

KEY RESEARCH ACCOMPLISHMENTS

- Continued patient enrollment, and delay from year 1 partially corrected.
- Initial data received back from biomarker discovery experiments.
- Discovery cohort database cleaning ongoing.
- Discovery cohort samples sent for massively-plexed genome wide discovery experiments.
- 5 abstracts/posters presented at ASA.
- Follow-up intervention study funded with start date of 30 September 2012 (T Buchheit PI).
- 2 papers accepted and published.

REPORTABLE OUTCOMES

Abstracts Presented at ASA Conference 2012

1. Pre-operative dexamethasone decreases the development of chronic mechanical allodynia in a mouse tibial spared nerve injury model.

2. Sub-anesthetic ketamine prior to nerve lesion reduces the development of chronic neuropathic pain in a mouse tibial spared nerve injury model.

3. Pain candidate pathway prioritization using interspecies plasma metabolomics.

4. Veterans Integrated Pain Evaluation Research (VIPER): Post-amputation Pain Phenotypes in Injured Military Service Personnel.

5. Veterans Integrated Pain Evaluation Research (VIPER): Pilot Cohort – Feasibility of Studying Combat Amputation Pain.

Posters Presented at ASA Conference 2012

1. Dexamethasone Attenuates Neuropathic Pain Behavior

2. Ketamine Attenuates Neuropathic Pain Behavior

3. Interspecies Plasma Metabolomics – Candidate Pain Pathway Prioritization

4. Veterans Integrated Pain Evaluation Research (VIPER): Post-amputation Pain Phenotypes in Injured Military Service Personnel.

5. Veterans Integrated Pain Evaluation Research (VIPER): Pilot Cohort – Feasibility of Studying Combat Amputation Pain.

Database

The secure, web-based application, named REDCap, chosen to support *data capture* for this project has been update to include changes approved on October 9 by WRNMMC IRB.

Publications

1. Genetics and epigenetics in perioperative medicine. Bain CR, Shaw AD. Curr Opin Crit Care. 2012 Oct; 18(5):548-54. PMID: 22914427 (PubMed-in process).

2. Epigenetics and the transition from acute to chronic pain. Buchheit T, Van de Ven T, Shaw, A. Pain Med. 2012 Nov; 13(11): 1474-90. Epub 2012 Sep 14. PMID: 22978429 (PubMed – in process).

Research Opportunity Applied for and Received

Dr. Thomas Buchheit, Co-Investigator on this project, submitted a proposal in response to the Department of Defense Program Announcement, Psychological Health/Traumatic Brain Injury Research Program, Funding Opportunity Number W81XWH-11-PHTBI-ANRA, submitted on January 6, 2012. He

received Notice of Award and his project titled "Regional Anesthesia and Valproate Sodium for the Prevention of Chronic Post-Amputation Pain" started September 30, 2012, a four-year project.

CONCLUSION

We have continued to make good progress with this project and are now entering the phase where data is being rapidly generated. We have identified some surprising biology in the form of lipid deregulation (metabolomic analyses) and are awaiting confirmation of these results in the rest of our discovery patients. We anticipate some exciting results as we receive our initial genetic and epigenetic data back this year, and are now seeking funding announcements that we may reach out to for continuation of this important work.

APPENDICES

Abstracts (5)

Posters (5)

Database - REDCap

Publications (2)

Research Opportunity Applied for and Received – Dr. Thomas Buchheit

ASA Abstract 1

Title: Pre-operative dexamethasone decreases the development of chronic mechanical allodynia in a mouse tibial spared nerve injury model.

AUTHOR(S):
T. Van de ven[1], H. Hsia[1], T. Buchheit[1], H. Sheng [1], A. D. Shaw[1]
1. Duke University, Durham, NC

Background:

Patients undergoing certain surgical procedures, such as thoracotomy or amputation, are at high risk for the development of chronic neuropathic pain. A large percentage of patients undergoing these major surgical procedures continue to have pain at the surgical site one year following the procedure and current therapy is limited.

New research suggests that pro-inflammatory responses to nerve injury play an important role in the development of chronic neuropathic pain. After peripheral nerve injury, macrophages, neutrophils, lymphocytes and mast cells infiltrate the injured nerve and release inflammatory mediators which cause further damage and can sensitize nociceptive receptors leading to peripheral and central sensitization.[3] Given the immunomodulatory and anti-inflammatory properties of dexamethasone, it deserves further research as a candidate drug for prevention of the development of chronic neuropathic pain. For this study, a spared nerve injury (SNI) mouse model was used to test dexamethasone as a therapeutic and preventative intervention in the development of chronic mechanical allodynia. [1,2]

Methods:

After IACUC approval, 30 C57/Bl6 mice were divided into three groups. 5 mice were used as the nerve injury control group and received a spared tibial nerve injury without pharmacological intervention. 5 mice were used as sham controls in which dissection down to the sciatic nerve and subsequent branches was accomplished, but no ligation and transection of nerves was performed. 5 mice were used as the experimental intervention group where intraperitoneal dexamethasone (10mg/kg) was administered 1 hour prior to surgical ligation and transection of the sural and common peroneal nerve with sparing of the tibial nerve. All surgery was performed under isoflurane anesthesia.

All mechanical threshold testing, including baseline and all subsequent post-surgical measurements, was performed with an electronic von Frey anesthesiometer (Life Science 2390 series). Baseline mechanical threshold testing was performed prior to surgery. Post-surgical measurements were performed starting on post-operative day 3 and every following third day, finishing on post-operative day 21.

Results:

Out of the three groups, only the SNI group demonstrated a statistically significant decrease in mechanical paw withdrawal thresholds on the operative side and maximal effect was observed on day 15. For the SNI group; ipsilateral compared to contralateral paw withdrawal thresholds: 1.21g +/-

0.127g vs. 5.32g +/- 1.21g, P = 0.041, respectively. For the sham control group; ipsilateral compared to contralateral paw withdrawal thresholds: 5.15g +/- 0.25g vs. 4.82g +/- 0.34g, P = 0.251, respectively. For the dexamethasone experimental group; ipsilateral compared to contralateral paw withdrawal thresholds: 5.392 +/- 0.299 vs. 5.496g +/- 0.76g, P = 0.784.

Conclusion:

Given these results, dexamethasone likely prevents the development of chronic mechanical allodynia by suppressing inflammatory processes leading to peripheral and central sensitization.

References:

1. Shields S, Eckert W, Basbaum A. *Spared Nerve Injury Model of Neuropathic Pain in the Mouse: A Behavioral and Anatomic Analysis*. The Jornal of Pain, Vol. 4, No 8: pp 465-470
2. Mogil J, Graham A, Ritchie J, Hughes S, Austin J, Schorscher-PetCu A, Bennett G. *Hypolocomotion, asymmetrically directed behaviors (licking, lifting, flinching, and shaking) and dynamic weight bearing (gait) changes are not measures of neuropathic pain in mice*. Molecular Pain 2010, 6:34. http://www.molecularpain.com/content/6/1/34
3. Bastos L, Medeiros D, Vieira R, Watkins L, Coelho M, Moraes M. *Intraneural dexamethasone applied simultaneously to rat sciatic nerve constriction delays the development of hyperalgesia and allodynia*. Neuroscience Letter 510 (2012) 20-23

ASA Abstract 2

Title: Sub-anesthetic ketamine prior to nerve lesion reduces the development of chronic neuropathic pain in a mouse tibial spared nerve injury model.

AUTHOR(S):
T. Van de ven[1], H. Hsia[1], T. Buchheit[1], H. Sheng [1], A. D. Shaw[1]
1. Duke University, Durham, NC

Background:

A common complication of nerve injury is the development of neuropathic pain. Patients undergoing surgical procedures, especially those requiring a large incision or amputation, are at high risk for the development of chronic neuropathic pain. A large percentage of patients undergoing these major surgical procedures continue to have pain at the surgical site one year following the procedure and current therapy is limited. The purpose of this study is to utilize a spared nerve injury (SNI) mouse model to test ketamine as a therapeutic and preventative intervention in the development of chronic neuropathic pain. This spared nerve model for neuropathic pain has been previously validated by prior research.[1]

NMDA receptor activity is thought to play a major role in central sensitization involved in the development of chronic neuropathic pain.[2] Ketamine, given as an anesthetic dose in a mouse SNI model, prevents the development of changes in mechanical thresholds.[1] This study postulated that a sub-anesthetic dose prior to SNI would also prevent the development of changes in mechanical threshold associated with a neuropathic pain phenotype.

Methods:

After IACUC approval, 25 C57/Bl6 mice were divided into three groups. 5 mice were used as the nerve injury control group and received a spared tibial nerve injury without pharmacological intervention. 5 mice were used as sham controls in which dissection down to the sciatic nerve and subsequent branches was accomplished, but no ligation and transection of nerves was performed. 5 mice were used as the experimental intervention group where subcutaneous ketamine (20mg/kg) was administered in between the shoulder blades 1 hour prior to surgical ligation and transection of the sural and common peroneal nerve with sparing of the tibial nerve. All surgery was performed under isoflurane anesthesia.

All mechanical threshold testing, including baseline and all subsequent post-surgical measurements, was performed with an electronic von Frey anesthesiometer (Life Science 2390 series). Baseline mechanical threshold testing was performed prior to surgery. Post-surgical measurements were performed starting on post-operative day 3 and every following third day, finishing on post-operative day 21.

Results:

Out of the three groups, only the SNI group demonstrated a statistically significant decrease in mechanical paw withdrawal thresholds on the operative side and maximal effect was observed on post-

operative day 15 (POD 15). For the SNI group; ipsilateral compared to contralateral paw withdrawal thresholds: 1.21g +/- 0.127g vs. 5.32g +/- 1.21g, P = 0.041, respectively on POD 15. For the sham control group; ipsilateral compared to contralateral paw withdrawal thresholds: 5.15g +/- 0.25g vs. 4.82g +/- 0.34g, P = 0.251, respectively on POD 15. For the ketamine experimental group; ipsilateral compared to contralateral paw withdrawal thresholds: 4.512g +/- 0.637g vs. 5.36g +/- 0.955g, P = 0.13 on POD 15.

Conclusion:

Given these results, a sub-anesthetic dose of ketamine 20mg/kg prior to spared nerve injury likely attenuates or prevents the development of mechanical allodynia most likely through NMDA receptor antagonism, which inhibits central sensitization.

References:

1. Shields S, Eckert W, Basbaum A. *Spared Nerve Injury Model of Neuropathic Pain in the Mouse: A Behavioral and Anatomic Analysis*. The Jornal of Pain, Vol. 4, No 8: pp 465-470
2. Zhou H, Chen S, Pan H. *Targeting N-methyl-D-aspartate receptors for treatment of neuropathic pain*. Expert Rev Clin Pharmacol. 2011 May 1; 4(3): pp 379-388

American Society of Anesthesiologists

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Control/Tracking Number : 12-A-3680-ASAHQ
Activity :Abstract
Current Date/Time : 3/27/2012 10:03:30 AM
TITLE:
Pain candidate pathway prioritization using interspecies plasma metabolomics.

AUTHOR(S):
A. D. Shaw[1], H. Hsia[1], T. Van de ven[1], T. Buchheit[1], J. Lucas[1], M. McDuffie[2], C. Buckenmaier[2];
[1]Duke University, Durham, NC, [2]WRNMMC, Bethesda, MD.

AFFIRMATIONS:

Affirmations (Complete):
*: I agree to the above statements
*: I agree to the above statements
*: Accept
*: Yes
*: Yes
*: Yes, I have IRB or IACUC approval
*: I agree to the above statements

SESSION CATEGORY:
3.1 CHRONIC AND CANCER PAIN - Basic Science

QUESTIONNAIRE:

Questionnaire (Complete):
Please select: No, do not consider my abstract for the 2012 ANESTHESIOLOGY Journal Symposium
Please select: The presenting author is NOT a resident or fellow.
Will you be able to participate in the Resident Research Forum to be held on Saturday, October 15th at 1:00PM? Not applicable
Funding Information: CDMRP award #DM102142 to Dr Shaw

ABSTRACT:
Background: Persistent pain after surgical nerve damage is a significant problem, affecting patients undergoing many different procedures. The biological pathways responsible are poorly characterized, and little progress has been made in the field of novel analgesic development. In order to prioritize the biological pathways of relevance we have compared the plasma metabolomes of humans with persistent pain after surgical amputation and C57/Bl6 mice undergoing spared nerve injury. We hypothesize that pathways that are demonstrably important in *both* species represent high priority candidates both for further mechanistic study, and also for therapeutic target discovery.
Methods: After IRB and IACUC approval we are studying 20 human subjects with persistent neuropathic pain who had sustained a traumatic amputation in the prior 3-18 months, and 20 C57/Bl6 mice who underwent sciatic spared nerve injury in the prior 3 weeks. Human chronic pain phenotypes were adjudicated by committee, mice chronic pain phenotypes were measured using electronic Von Frey

apparatus and plasma samples were drawn for metabolomic analysis from both humans and mice. Assays are conducted by Metabolon Inc, Raleigh, NC. Data are compared in order to identify pathways of relevance that are either convergent across both species, or show significant divergence between humans and mice. In general, metabolite fold increase or decrease is compared between human and mouse phenotypes using 2-way ANOVA, and multiple comparisons controlled using false discovery. Dimensionality reduction is achieved using principal components analysis.
Results: Metabolomic analysis identifies over 300 different metabolites, and many more unknown compounds. Some biochemical pathways are convergent between humans and mice, whereas others are restricted to a single species. Data will be shown using a heatmap (fold change) diagram, and annotated Venn diagrams of overlapping pathway significance.
Conclusions: We are conducting comparative biological investigations of persistent pain phenotypes in two evolutionarily distant species in order to detect pathways of biological relevance following peripheral nerve injury. We will use these data to inform further proteomic and genomic experiments probing ever deeper into the preserved, but maladapted, inflammatory response to nerve injury.

SUMMARY:
We present a comparative biological study of the human and murine plasma metabolomic response to peripheral nerve injury.

Status: Complete

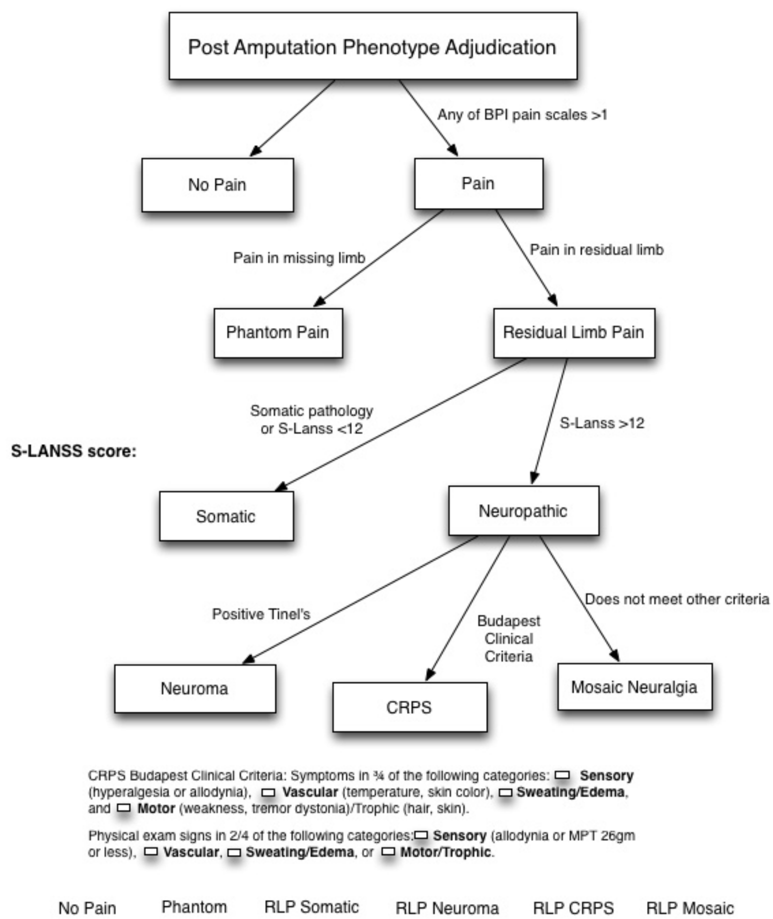
Veterans Integrated Pain Evaluation Research (VIPER): Post-amputation Pain Phenotypes in Injured Military Service Personnel

Thomas Buchheit MD, Thomas VandeVen MD PhD, Mary McDuffie RN, Hung-Lun John Hsia MD, COL Chester "Trip" Buckenmaier MD, and Andrew Shaw MB FRCA

Background

Post-amputation pain is present in more than 50% of injured military service members after amputation.[1] Although distinct pain syndromes such as neuroma and complex regional pain syndrome have been described[2,3], most studies discriminate only between phantom and residual limb pain.[4] Similar to advances that have been made with other chronic diseases after diagnostic improvements,[5] classifying pain phenotypes may ultimately lead to more disease-specific and effective therapies. With this goal, we are performing a collaborative study (Veterans Integrated Pain Evaluation Research (VIPER)) between Duke University and Walter Reed National Military Medical Center (WRNMMC) of injured military service personnel who have undergone previous traumatic amputation. We report the assessment and phenotypic adjudication of the first 15 patients enrolled in VIPER, who represent the initial pilot cohort.

METHODS



After IRB approval, the VIPER pilot clinical cohort was assessed using several well established and validated questionnaire instruments including the Brief Pain Inventory (BPI), Self-Reported Leeds Assessment of Neuropathic

Symptoms and Signs Pain Scale (S-LANSS), Complex Regional Pain Syndrome questions (Budapest Clinical Criteria) phantom and residual limb pain questionnaires.

These questionnaire instruments were applied to each case as part of a formal endpoint adjudication process as required by the VIPER protocol in order to discriminate between distinct pain phenotypes. Using an algorithm previously reported by our group [4], phantom pain was first distinguished, and subsequently, residual limb pain was sub-categorized into 1) Somatic 3) Neuroma/Neuritis 4) CRPS or 5) Mosaic Neuralgia (neuropathic pain not otherwise specified).

RESULTS

Using these validated assessment tools, we were able to successfully discriminate between multiple categories of post-amputation pain in this preliminary cohort. We found that 86% described phantom pain, 13% noted residual limb somatic pain, 33% residual limb neuroma pain, 7% residual limb CRPS pain, and 20% described neuralgic limb pain not otherwise specified (Mosaic neuralgia). Importantly, there was significant overlap between phantom limb pain and residual limb neuralgic pain.

Patient	Chronic Pain	Phantom Pain	Residual Limb Pain			
			RLP Somatic	RLP Neuroma	RLP CRPS	RLP Mosaic
1	yes	yes		yes		
2	yes	yes	yes			
3	yes			yes		
4	yes	yes				yes
5	yes	yes		yes		
6	yes	yes				yes
7	no					
8	yes	yes			yes	
9	no					
10	yes	yes	yes			
11	no					
12	yes	yes		yes		
13	yes	yes				
14	yes	yes		yes		
15	yes	yes				yes

Summary

This preliminary research describes significant phenotypic complexity within the post-amputation pain syndromes, including several different subtypes of residual limb neuropathic pain. Further cohort analyses will allow for better diagnostic discrimination between post-amputation pain subtypes, and may facilitate targeted future therapies.

1. Reiber GE, McFarland LV, Hubbard S, et al. Servicemembers and veterans with major traumatic limb loss from Vietnam war and OIF/OEF conflicts: survey methods, participants, and summary findings. *J Rehabil Res Dev.* 2010;47(4):275-297.
2. Sehirlioglu A, Ozturk C, Yazicioglu K, Tugcu I, Yilmaz B, Goktepe AS. Painful neuroma requiring surgical excision after lower limb amputation caused by landmine explosions. *International orthopaedics.* Apr 2009;33(2):533-536.
3. Isakov E, Susak Z, Korzets A. Reflex sympathetic dystrophy of the stump in below-knee amputees. *The Clinical journal of pain.* Sep 1992;8(3):270-275.
4. Lindsay DR, Pyati S, Buchheit TE, Shaw A. Residual limb pain: more than a single entity? *Anesthesiology.* Jan 2012;116(1):224.
5. Jaglowski S, Jones JA. Choosing first-line therapy for chronic lymphocytic leukemia. *Expert Rev Anticancer Ther.* Sep 2011;11(9):1379-1390.

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TITLE:
Veterans Integrated Pain Evaluation Research (VIPER) Pilot Cohort: Feasibility of Studying Combat Amputation Pain

AUTHOR(S):
A. D. Shaw[1], T. Buchheit[1], T. Van de ven[1], H. Hsia[1], M. McDuffie[2], C. Buckenmaier[2];
[1]Duke University, Durham, NC, [2]WRNMMC, Bethesda, MD.

AFFIRMATIONS:

Affirmations (Complete):
- *: I agree to the above statements
- *: I agree to the above statements
- *: Accept
- *: Yes
- *: No animal subjects were involved in the research
- *: Yes, I have IRB or IACUC approval
- *: I agree to the above statements

SESSION CATEGORY:
3.2 CHRONIC AND CANCER PAIN - Clinical

QUESTIONNAIRE:

Questionnaire (Complete):
Please select: No, do not consider my abstract for the 2012 ANESTHESIOLOGY Journal Symposium
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Funding Information: CDMRP award #DM102142 to Dr Shaw

ABSTRACT:
Veterans Integrated Pain Evaluation Research (VIPER) Pilot Cohort:Feasibility of Studying Combat Amputation Pain
Andrew Shaw MB FRCA, Thomas Buchheit MD, Thomas VandeVen MD PhD, Mary McDuffie RN, Hung-Lun John Hsia MD, Chester Buckenmaier MD
BACKGROUND
We are currently enrolling military service personnel returning from Operation Iraqi Freedom (OIF), Operation Enduring Freedom (OEF) and Operation New Dawn (OND) in a study of the determinants of persistent pain after devastating limb injury in the absence of traumatic brain injury. To date (March 2012) we have enrolled 20 soldiers (the VIPER pilot cohort), who underwent devastating peripheral limb injury

between 3 and 18 months previously, and here we report data regarding the characteristics of their pain syndromes. This cohort represents 13% of the total planned enrollment of 150 patients.

METHODS

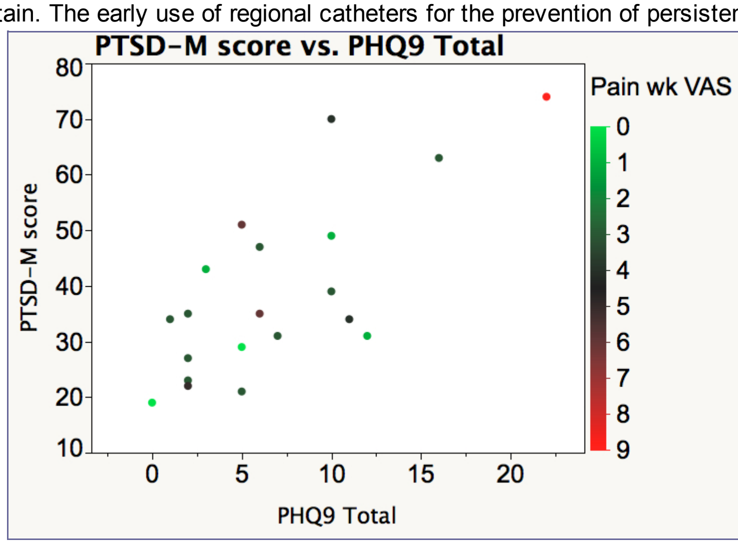
After IRB approval, the VIPER pilot clinical cohort was assessed using several well established and validated questionnaire instruments including the Brief Pain Inventory (BPI), Self-Reported Leeds Assessment of Neuropathic Symptoms and Signs Pain Scale (S-LANSS), Post Traumatic Stress Disorder (Military) scale, phantom and residual limb pain questionnaires. Additionally, soldiers were interviewed regarding their pain symptomatology, their pain perceptions, and measurements made of their mechanical detection and pain thresholds. Descriptive data are reported for all 20 patients; no comparison data are reported because of the small sample size and the fact that this pilot cohort primarily represents a study of the feasibility of enrolling these soldiers as they rehabilitate from devastating combat injury. Blood samples for biomarker and therapeutic discovery studies have also been collected, details of which are reported in a separate abstract.

RESULTS

18 of 20 (90%) patients suffered lower limb amputation and 2 of 20 (10%) upper limb amputation. 17 of 20 (85%) patients reported phantom pain, and 15 of 20 (75%) reported residual limb (stump) pain. 7 patients reported that these symptoms interfere significantly with their daily activities. Mean (SD) VAS score across the whole cohort was 3.2 (2.2), mean neuropathic score was 13.6 (7.7), mean PTSD score was 38.9 (15.9) and mean PHQ 9 score was 6.9 (5.6). There was no statistically significant effect of regional anesthetic catheter use, age, ethnicity, race, smoking status, type of injury, type of amputation or body mass index on either the incidence or severity of pain; however the p value for regional catheter use was 0.06 suggesting that as the sample size increases this variable may develop significance. The figure shows PTSD, PHQ 9 and VAS scores for all 20 patients.

Summary

In this pilot cohort of 20 patients who sustained devastating peripheral limb combat injuries, the incidence of chronic phantom and/or residual limb pain was greater than 75%. There is considerable overlap between phantom and residual limb pain, and both interfere significantly with patients' daily activities. The incidence of PTSD symptomatology is high in this military population; but whether or not this is important for the pain subtype is presently uncertain. The early use of regional catheters for the prevention of persistent pain in the traumatic amputation setting may warrant further study.



SUMMARY:

We report the initial 20 patient pilot cohort of an ongoing study of the clinical and molecular determinants of persistent pain after combat amputation.

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Dexamethasone Attenuates Neuropathic Pain Behavior

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Department of Anesthesiology, Duke University Medical Center/Durham VAMC, Durham, NC USA

Introduction

- Neuropathic pain is a common complication of nerve injury.
- Proposed mechanisms include both local and systemic inflammation.
- Dexamethasone is a known anti-inflammatory agent often used in the operating room.
- Plasma metabolomics are a useful cross-species pathway discovery tool.

Hypotheses

- Dexamethasone attenuates the development of neuropathic pain behavior
- Spared nerve injury and treatment with dexamethasone cause reproducible metabolic changes
- Differentially regulated metabolites can serve as biomarkers of pain susceptibility and can inform pathway discovery.

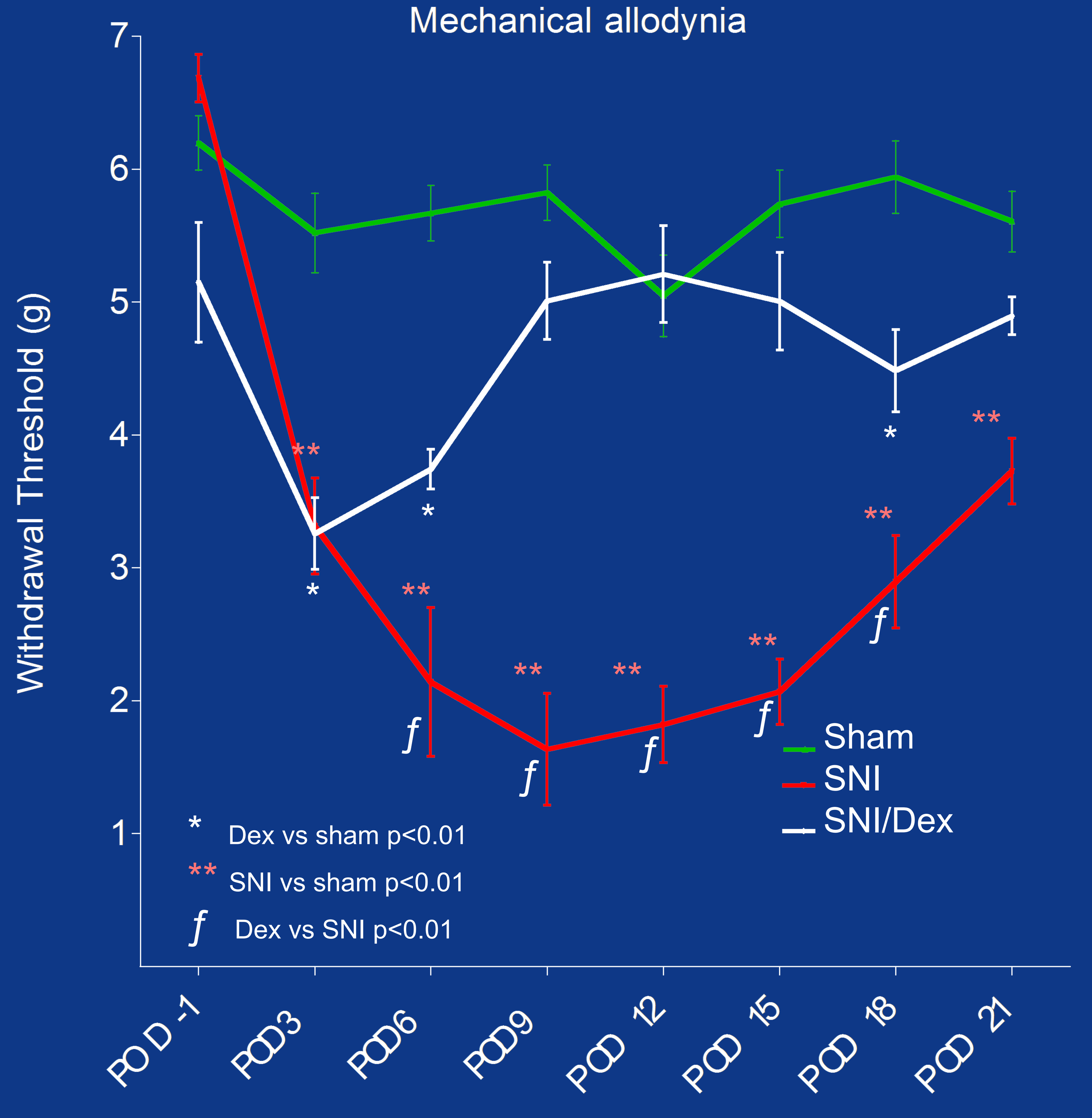

Figure 1: Analysis by two way ANOVA for time and treatment

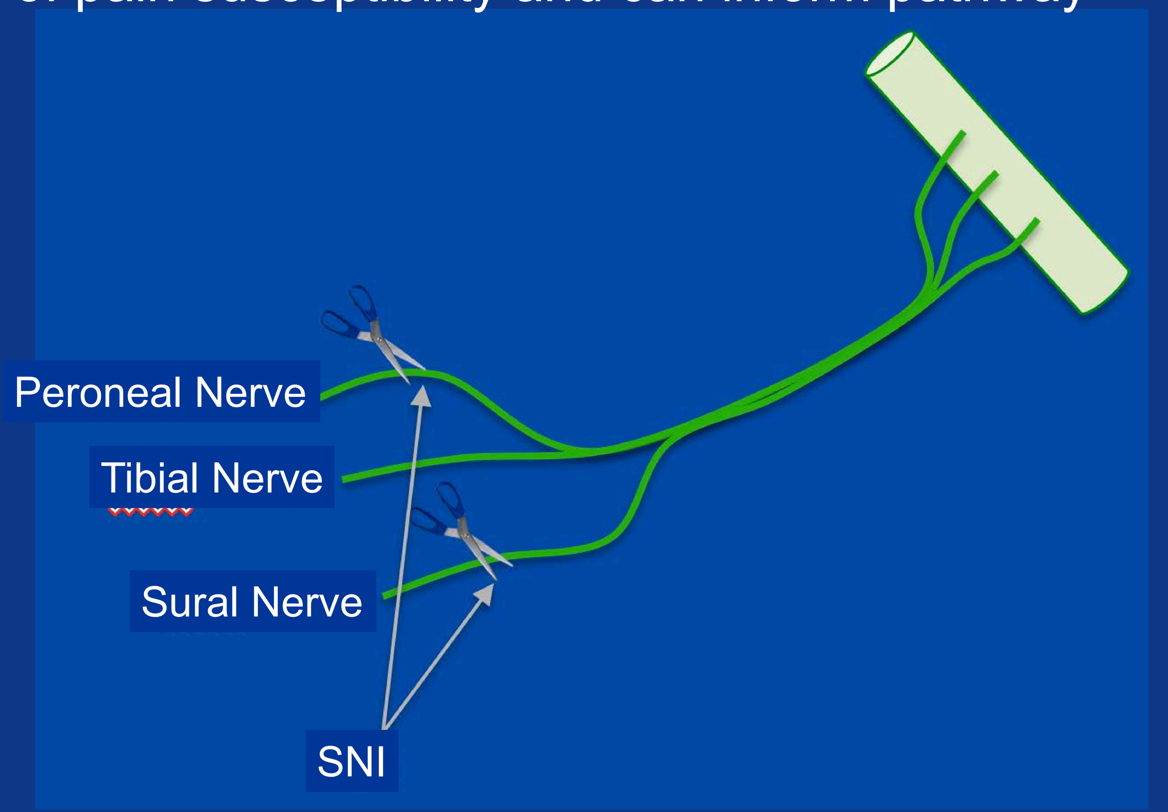

Figure 2: Spared Nerve Injury Model

Group	n	Description
Mouse CTRL	5	Sham
Mouse INT	15	SNI
Mouse TRT	5	SNI/Dex

Figure 3: Metabolomics Study Overview

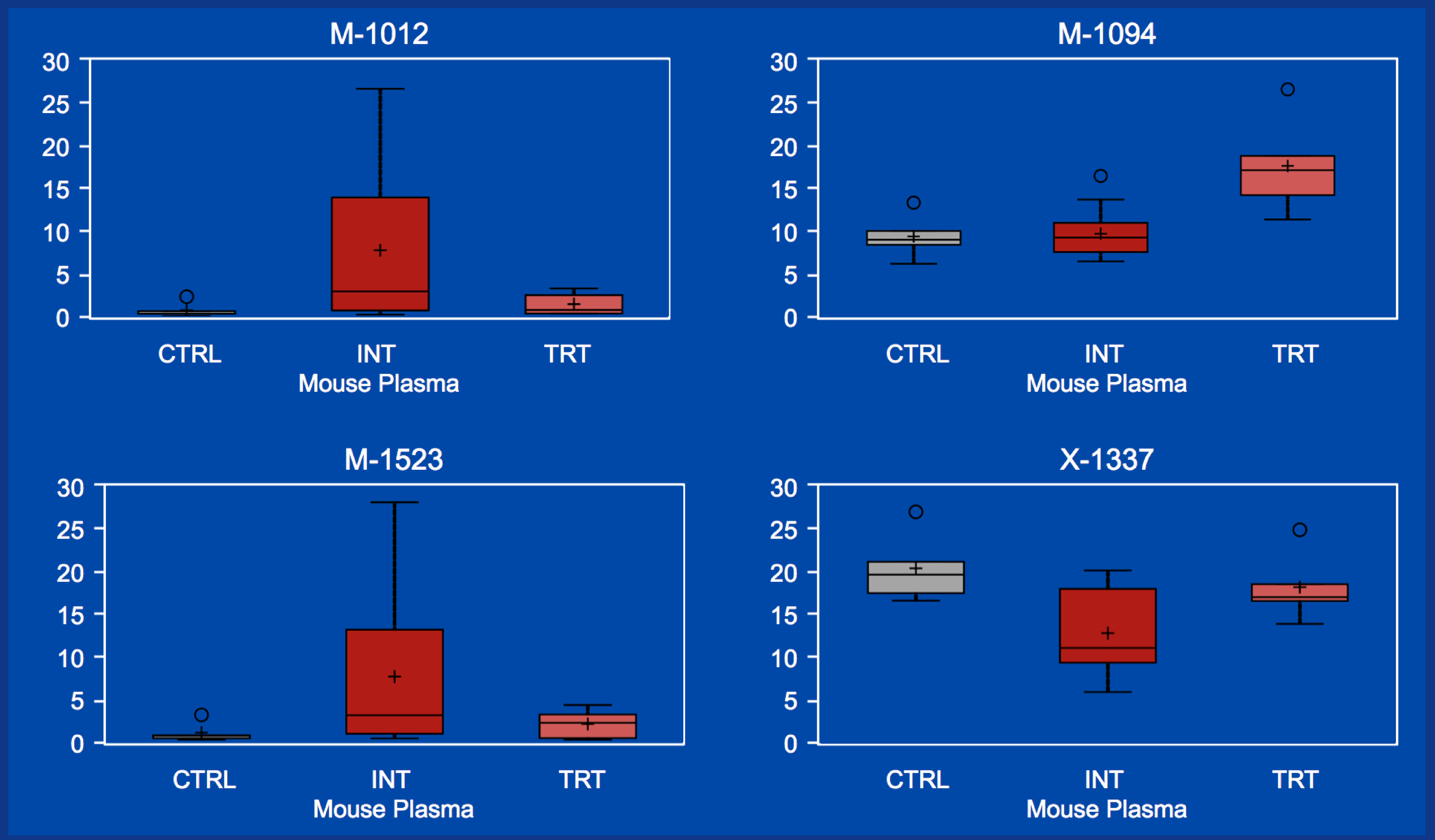

Figure 4: Metabolite concentration box plots

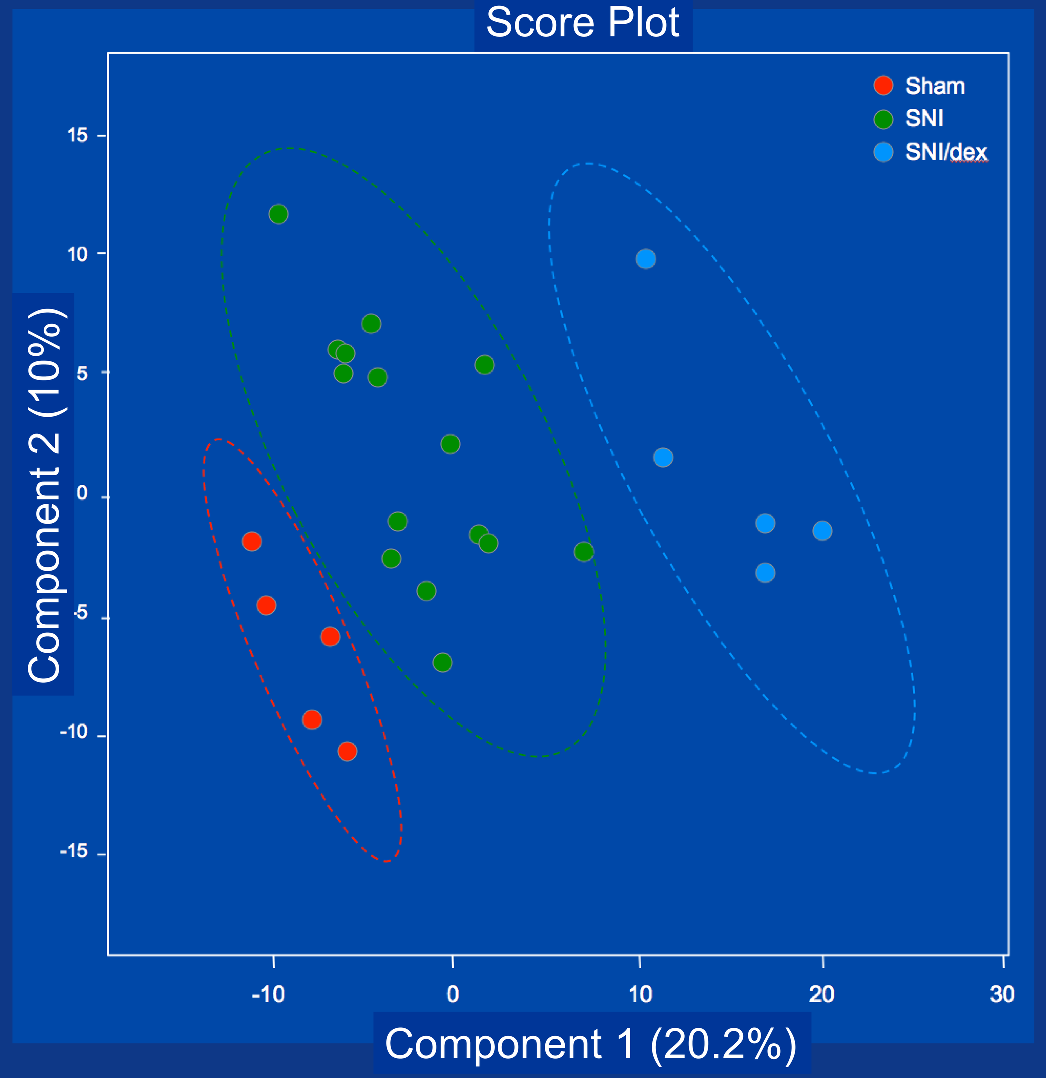

Figure 5: PLS-DA score plot showing class separation

Methods

- After IACUC approval, 30 C57/BI6 mice were randomly allocated into three groups:
  - Spared nerve injury (SNI), N=10
  - Sham surgery, N=10
  - Dexamethasone/SNI, N=10
- Mechanical withdrawal threshold was measured using an electronic von Frey anesthesiometer.
- Blood plasma was obtained and unbiased metabolomics performed at Metabolon, Inc.

Results

- The SNI group showed a statistically significant ($P < 0.01$) decrease in paw withdrawal threshold (PWT) on the surgical hind paw compared with sham.
- Compared to the SNI group, the treatment group demonstrated statistically significant ($P < 0.01$) increases in PWT.
- 51 metabolites are differentially regulated in sham vs. SNI operated mice. ($p < 0.05$ FDR corrected)
- 177 metabolites are differentially regulated between SNI and SNI/Dexamethasone treated mice. ($p<0.05$ FDR corrected)
- Multivariate analysis shows significant differential clustering of all three experimental groups

Conclusions

- Dexamethasone attenuates neuropathic pain behavior.
- SNI and dexamethasone treatment produce reproducible metabolic changes in mice.

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Supported by NIH T32 #2T32GM008600-16 and DMRDP Grant #DM102142

Ketamine Attenuates Neuropathic Pain Behavior

TJ Van de Ven, HL Hsia, H Sheng, D Macleod, TE Buchheit, AD Shaw
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Introduction

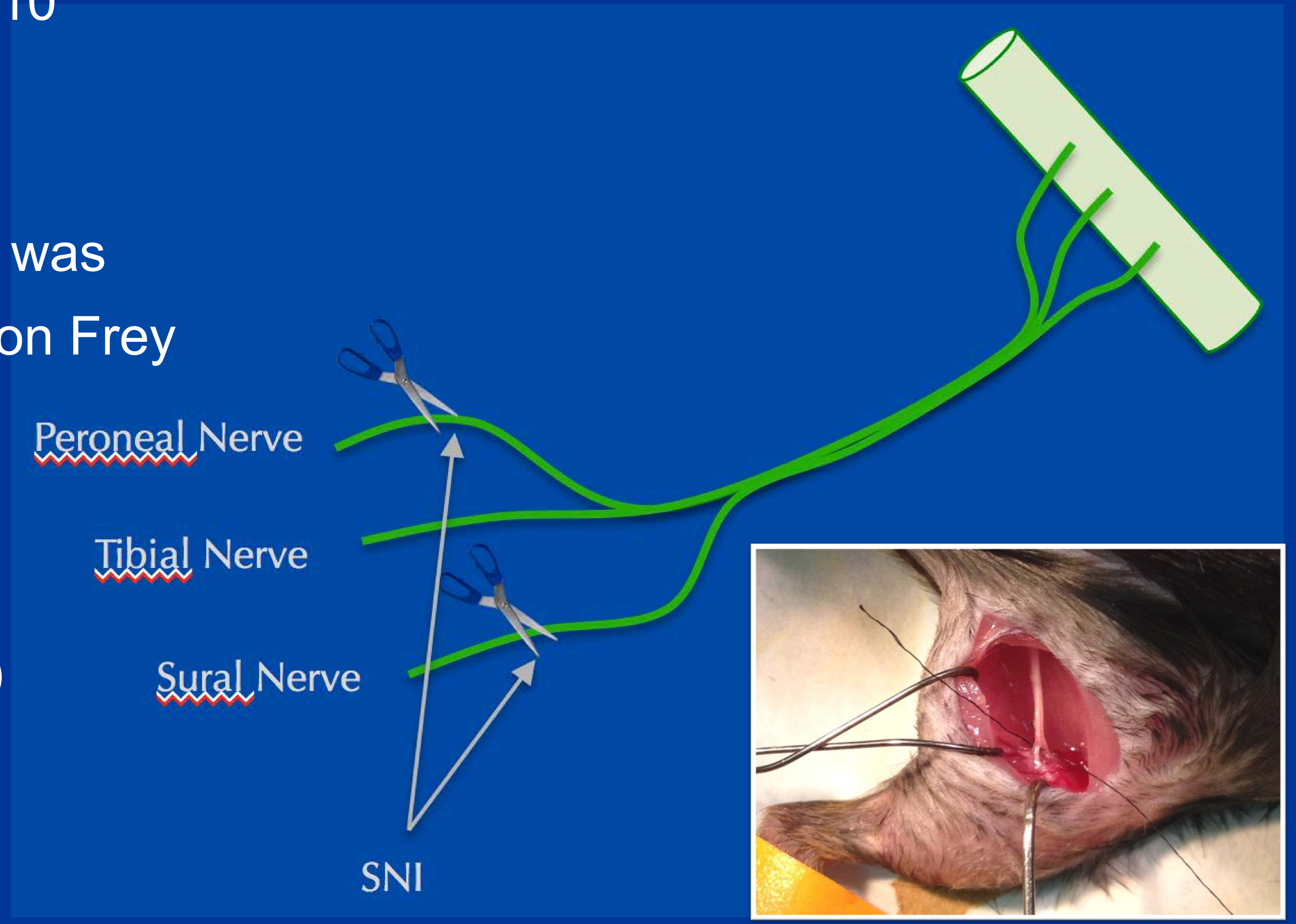
- Neuropathic pain is a common complication of nerve injury.
- Proposed mechanisms include NMDA receptor mediated central sensitization.
- It is unclear whether a preoperative subanesthetic dose of ketamine can prevent chronic pain

Hypothesis

- We hypothesized that ketamine would attenuate the development of neuropathic pain behavior in a mouse model.

Methods

- After IACUC approval, 30 C57/Bl6 mice were randomly allocated into three groups:
  - Spared nerve injury (SNI), N=10
  - Sham surgery, N=10
  - Ketamine/SNI, N=10
- Mechanical withdrawal threshold was measured using an electronic von Frey anesthesiometer.



Results

- The SNI group showed a statistically significant ($P < 0.01$) decrease in paw withdrawal threshold (PWT) on the surgical hind paw compared with sham.
- Compared to the SNI group, the treatment group demonstrated statistically significant ($P < 0.01$) increases in PWT.

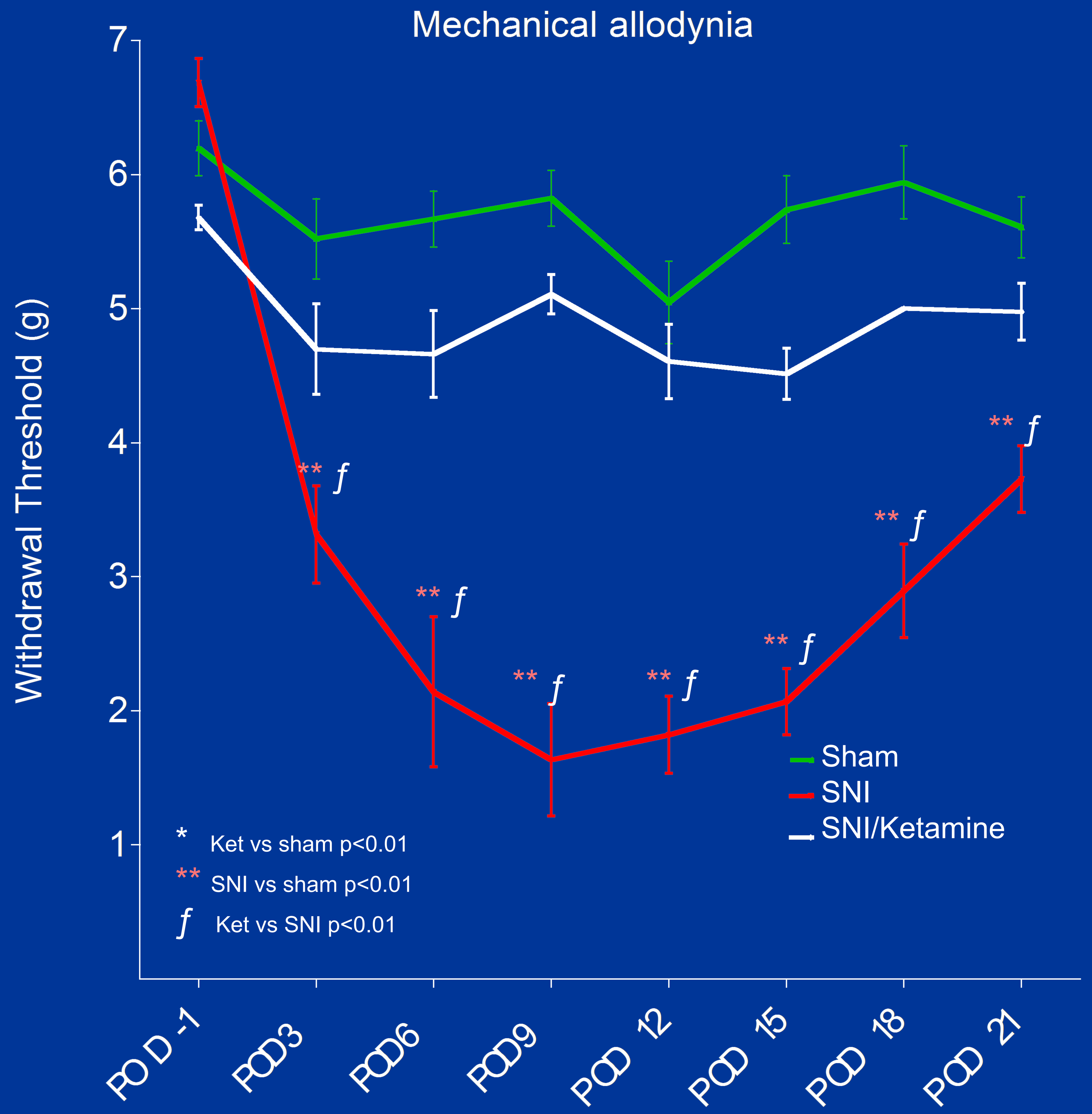


Figure: Withdrawal threshold vs post-operative day. Analysis by repeatedmeasures ANOVA with Tukey post-hoc analysis.

Conclusions

- A one-time, preoperative, subanesthetic dose of ketamine attenuates neuropathic pain behavior.

References
1. Shields S, Eckert W, Basbaum A. The Journal of Pain, Vol. 4, No 8: pp 465-470
2. Mogil J, Graham A, Ritchie J, Hughes S, Austin J, Schorscher-PetCu A, Bennett G. Molecular Pain 2010, 6:34.

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Interspecies Plasma Metabolomics - Candidate Pain Pathway Prioritization

Hung-Lun John Hsia MD, Thomas VandeVen MD PhD, Thomas Buchheit MD, Joseph Lucas PhD, Mary McDuffie RN, Chester Buckenmaier MD, and Andrew Shaw MB FRCA

Department of Anesthesiology, Duke University Medical Center, Walter Reed National Military Medical Center, Durham Veterans Affairs Medical Center

Background:

Persistent pain after surgical nerve damage is a significant problem, affecting patients undergoing many different procedures. The biological pathways responsible are poorly characterized, and little progress has been made in the field of novel analgesic development. In order to prioritize the biological pathways of relevance we have compared the plasma metabolomes of humans with and without persistent pain after surgical amputation and C57/Bl6 mice undergoing spared nerve injury. We hypothesize that pathways that are demonstrably important in both species represent high priority candidates for further mechanistic study and therapeutic target discovery.

Methods:

After IACUC approval, 30 C57/Bl6 mice were randomly allocated into three groups:

- Sham surgery, N=5
- Spared Nerve Injury (SNI), N=15
- Dexamethasone/SNI, N=5

Observation of greatest phenotypic difference (paw withdrawal threshold levels) occurred on POD 15. At that time plasma was drawn from all mice and flash frozen at -80C and sent off for metabolic analysis.

After IRB approval and acquired consent, fifteen patients were selected from the Veterans Investigative Pain Evaluation Research (VIPER) cohort group and allocated to two groups based on a formal ajudication process to differentiate clinical pain phenotypes. This process placed them into two distinct groups:

- Control group, N=9
- Case group, N=6

Both groups received surgical amputations. The case group consisted of patients with the most severe pain scores. In contrast, the control group exhibited the lowest pain scores.

Results:

In mouse, 583 metabolites were analyzed and quantified, consisting of 345 named and 238 unnamed biochemicals. In humans, 658 metabolites, consisting of 363 named and 295 unnamed biochemicals, were analyzed. Plot scores demonstrate clear metabolic profile separation between groups in both human and mouse.

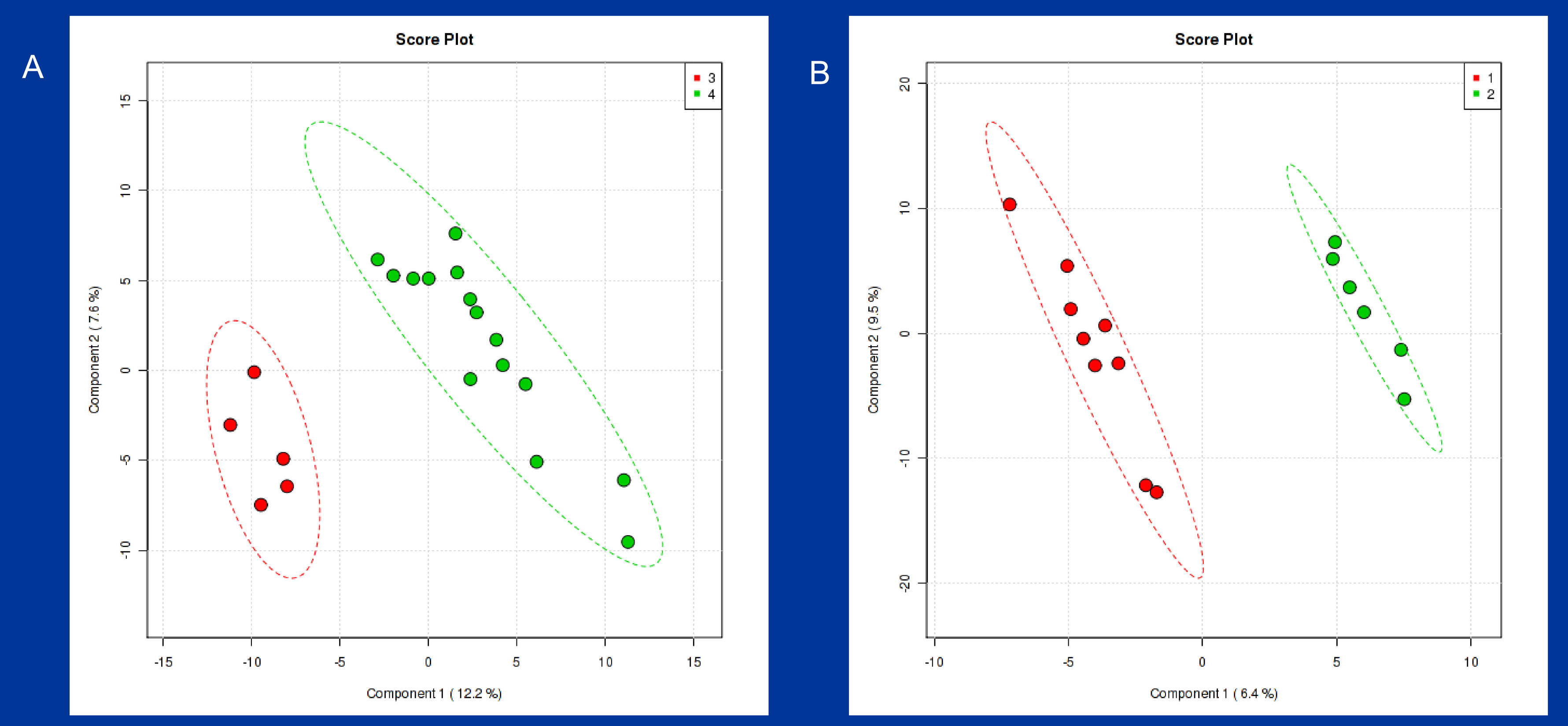

Fig 1: The figures above are score plots demonstrating the separation of differential metabolic profiles between groups in both mouse and humans. (A) Mouse sham surgery group (red dots) separated from nerve ligation group (green dots). (B) Human control group (red dots) separated from case group (green dots)

Statistical Comparisons Mouse Plasma				
ANOVA Contrasts	Total number of biochemicals with $p \leq 0.05$	Biochemicals (↑↓) $p \leq 0.05$	Total number of biochemicals with $0.05<p<0.10$	Biochemicals (↑↓) $0.05<p<0.10$
INT / CTRL	51	11 | 40	40	13 | 27
TRT / CTRL	183	75 | 108	34	16 | 18
TRT / INT	177	93 | 84	51	21 | 30
One Way ANOVA	Total number of biochemicals with $p \leq 0.05$		Total number of biochemicals with $0.05<p<0.10$	
Group Effect	184		43	

Table 1: One way ANOVA analysis demonstrating significant differences in biochemical species between groups

Welch's Two Sample t-Test Human Plasma	CASE / CTRL
Total number of biochemicals with $p \leq 0.05$	18
Biochemicals (↑↓)	7 | 11
Total number of biochemicals with $0.05<p<0.10$	27
Biochemicals (↑↓)	10 | 17

Table 2: Welch's Two Sample t-Test demonstrating significant differences between the two human groups

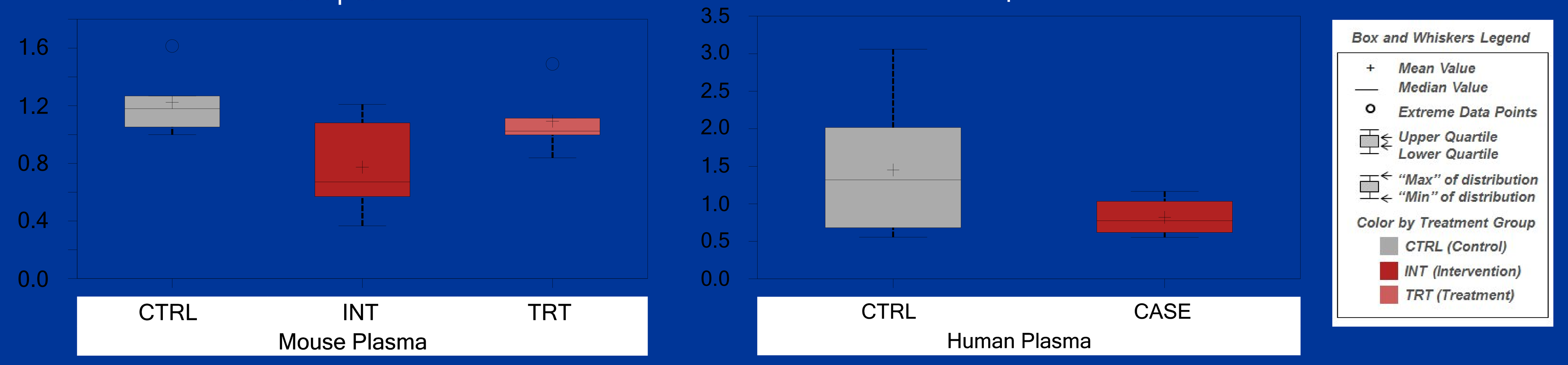

Fig 2: The graphs above demonstrate an identical biochemical species in both mouse and human which is down-regulated in nerve injury pain

Conclusions:

There were significant differences in the metabolic profiles between groups in both mouse and humans. Also, there is preserved cross-species differential expression of specific metabolic products.

References:

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Veterans Integrated Pain Evaluation Research (VIPER): Post-Amputation Pain Phenotypes in Injured Military Service Personnel

Thomas Buchheit MD, Thomas Van de Ven MD PhD, David MacLeod, MB FRCA, Mary McDuffie RN, Hung-Lun John Hsia MD, COL Chester "Trip" Buckenmaier MD, and Andrew Shaw MB FRCA

Departments of Anesthesiology, Duke University Medical Center, Walter Reed National Military Medical Center, and Durham Veterans Affairs Medical Center

Background

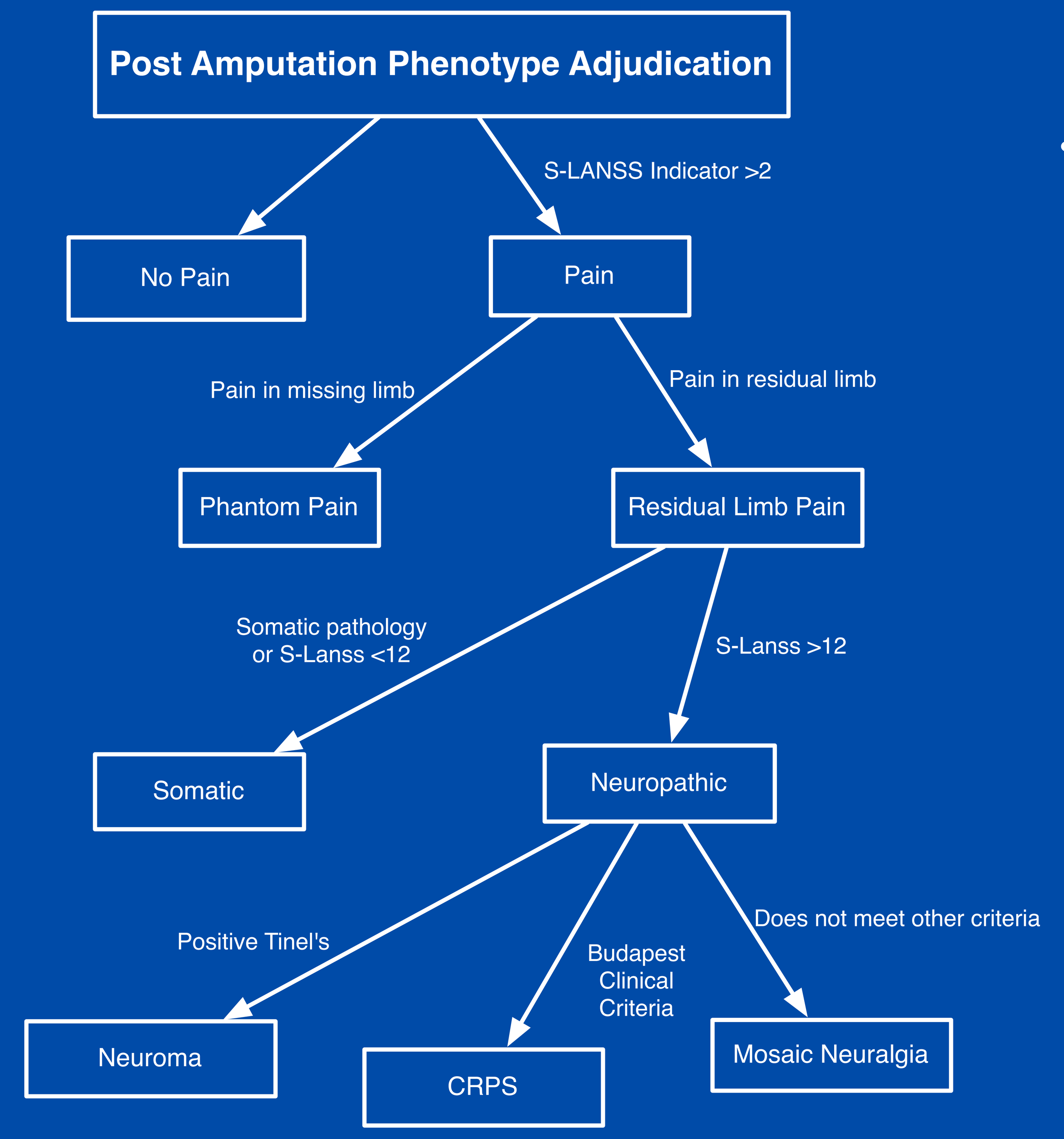
- Chronic pain is a common problem in injured military service members undergoing amputation.[1]
- Most studies of post-amputation pain only discriminate phantom and residual limb pain.[2]
- Sub-classification of pain phenotypes is a likely important step in the development of disease-specific therapies.
- A collaborative study (Veterans Integrated Pain Evaluation Research (VIPER)) between Duke University, Walter Reed National Military Medical Center (WRNMMC) and the Durham VAMC is being conducted to further define post-amputation clinical phenotypes and to correlate these findings with circulating biomarkers of persistent pain.
- Here we report on the initial cohort of 41 military service members who have undergone clinical assessment and phenotypic adjudication.

Methods

Phenotypic Assignment

After IRB approval, the VIPER clinical cohort was assessed using validated questionnaire instruments:

- Brief Pain Inventory (BPI)
- Self-Reported Leeds Assessment of Neuropathic Symptoms and Signs Pain Scale (S-LANSS)
- Complex Regional Pain Syndrome (Budapest Clinical Criteria)
- Phantom and residual limb pain questionnaires
- A formal endpoint adjudication was performed using the algorithm previously reported by our group[3]
  - Phantom and residual limb pain were discriminated.
  - Residual limb pain was then sub-categorized into a) Neuroma b) CRPS c) Mosaic Neuralgia or d) Somatic.

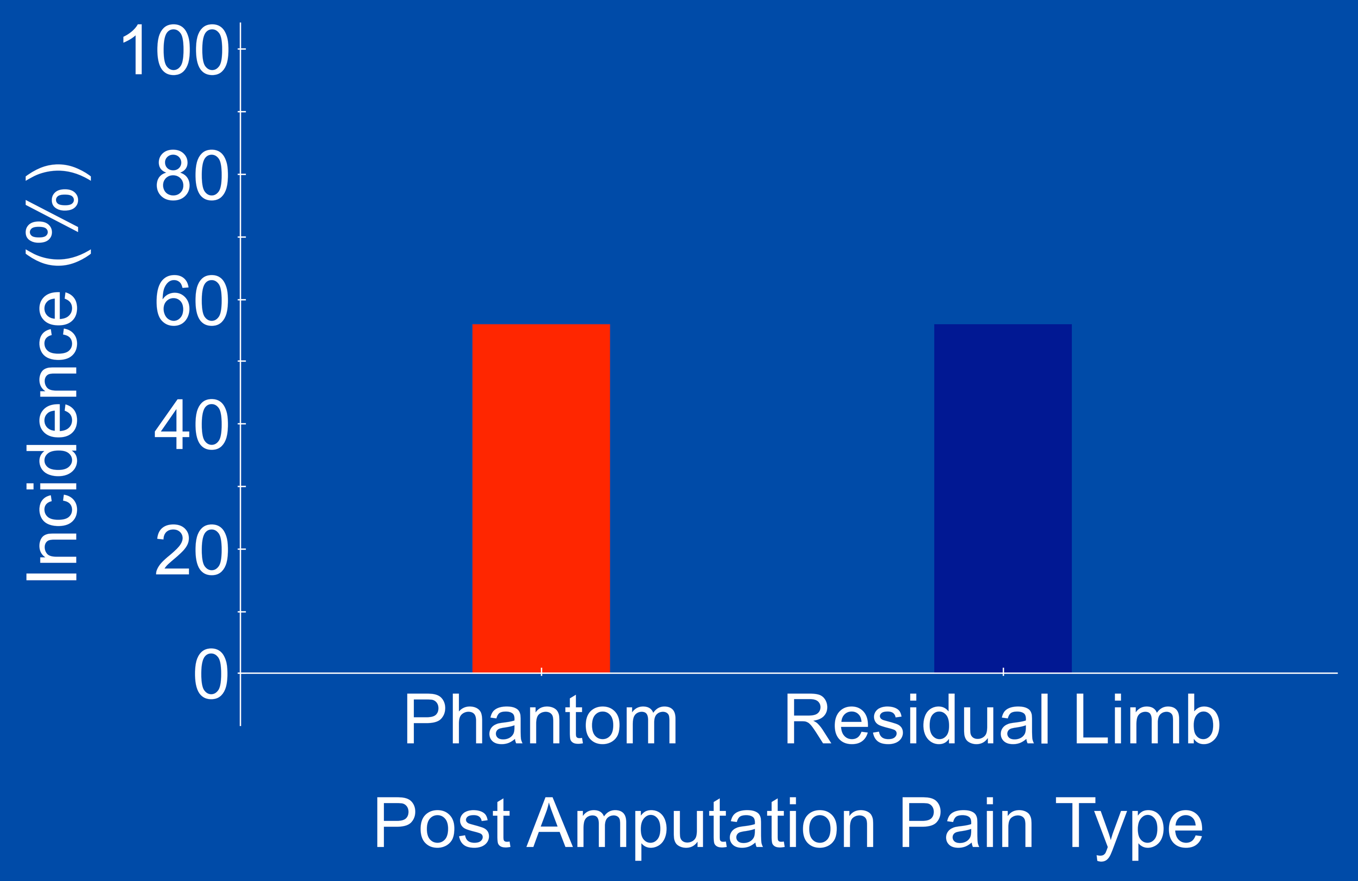


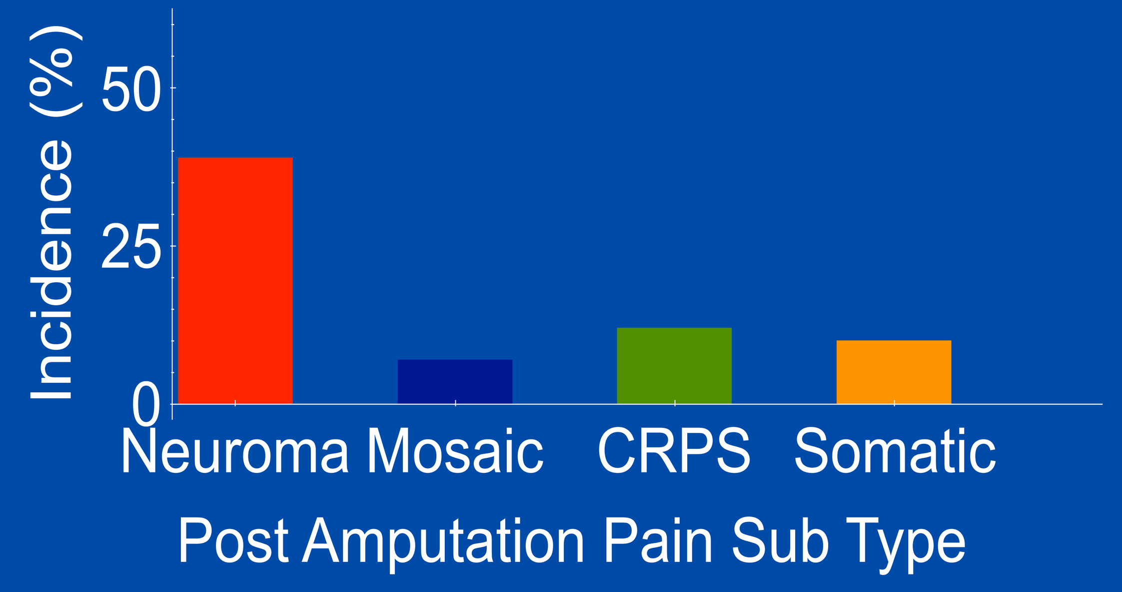
CRPS Budapest Clinical Criteria: Symptoms in ¾ of the following categories: ☐ **Sensory** (hyperalgesia or allodynia), ☐ **Vascular** (temperature, skin color), ☐ **Sweating/Edema**, and ☐ **Motor** (weakness, tremor dystonia)/Trophic (hair, skin).

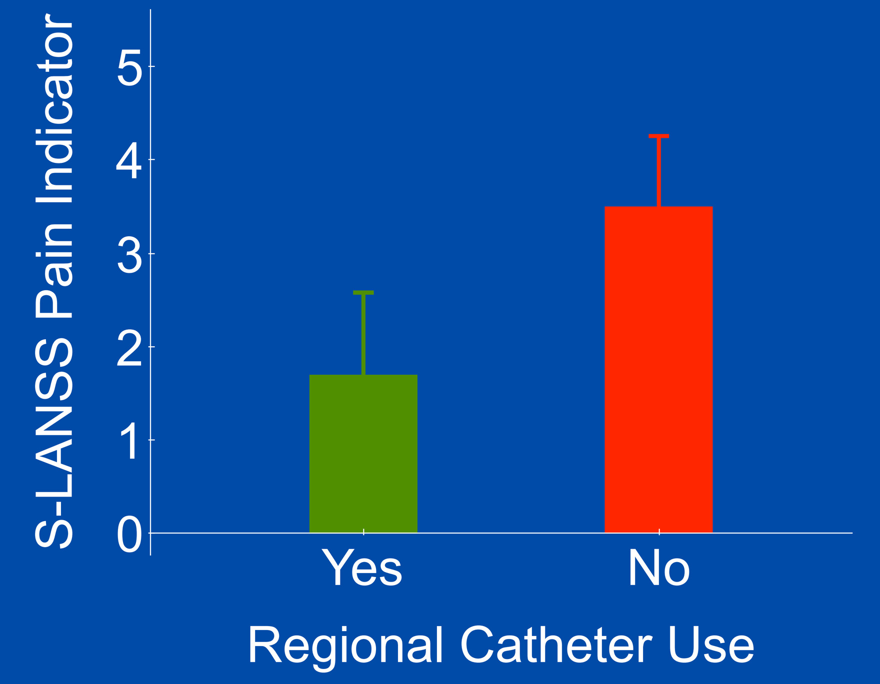
Physical exam signs in 2/4 of the following categories: ☐ **Sensory** (allodynia), ☐ **Vascular**, **Sweating/Edema**, or ☐ **Motor/Trophic**.

Results

- Using the Duke Post-Amputation Pain Algorithm (Duke PAPA), we discriminated between several post-amputation pain subtypes in this preliminary cohort of military service members.
- We found an overall incidence of post-amputation pain of 61%.
  - 56% described phantom pain
  - 56% described residual limb pain (RLP)
  - There was significant overlap with these diagnoses, but they did not always co-exist
- Of those subjects with RLP the following diagnostic categories were noted:
  - 70% neuroma
  - 22% CRPS
  - 13% Mosaic neuralgia (neuralgic pain not otherwise specified)
  - 17% somatic
- We additionally observed that the use of regional anesthesia catheters at the time of injury is associated with a decreased incidence of post-amputation pain during our assessment.
  - This effect appears secondary to reductions in residual limb pain, but not reductions in phantom pain.







Conclusions

- We observed phenotypic complexity of post-amputation pain symptoms in this initial cohort including:
  - Significant but not complete overlap in the diagnoses of phantom and residual limb pain
  - Several distinct subtypes of residual limb neuropathic pain
  - A predominant contribution of neuroma symptoms in service members with residual limb pain
- We additionally observed that the use of regional anesthesia catheters at the time of injury is associated with a decreased incidence of chronic pain during our assessment.

References

1. Reiber GE, McFarland LV, Hubbard S, et al. Servicemembers and veterans with major traumatic limb loss from Vietnam war and OIF/OEF conflicts: survey methods, participants, and summary findings. J Rehabil Res Dev. 2010;47(4):275-297.
2. Ephraim PL, Wegener ST, MacKenzie EJ, Dillingham TR, Pezzin LE. Phantom pain, residual limb pain, and back pain in amputees: results of a national survey. Arch Phys Med Rehabil. Oct 2005;86(10):1910-1919.
3. Lindsay DR, Pyati S, Buchheit TE, Shaw A. Residual limb pain: more than a single entity? *Anesthesiology.* Jan 2012;116(1):224.

Supported by DMRDP Grant #DM102142

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Veterans Integrated Pain Evaluation Research (VIPER) Pilot Cohort: Feasibility Of Studying Combat Amputation Pain

Andrew Shaw MB FRCA, Tom Buchheit MD, Tom Vandeven MD PhD, David Macleod MB FRCA, Mary McDuffie RN, Nancy Kwon RN, John Hsia MD, Mary Kirkley, Trip Buckenmaier MD

Departments of Anesthesiology, Duke University Medical Center, Durham, NC; Walter Reed National Military Medical Center, Bethesda, MD; Durham VA Medical Center, Durham, NC

Introduction

- We are studying combat amputation injury in OIF/OEF/OND personnel
- Our overarching goal is to identify novel biomarkers of amputation pain subtypes and better define the mechanisms involved in the transition from acute to chronic nerve injury pain
- Here we report summary clinical data from our pilot cohort

Patients

- 41 subjects with traumatic amputation have been enrolled since January 2012
- All sustained a traumatic injury leading to loss of a limb
- Data are collected between 3 and 18 months after initial injury in theater

Endpoints

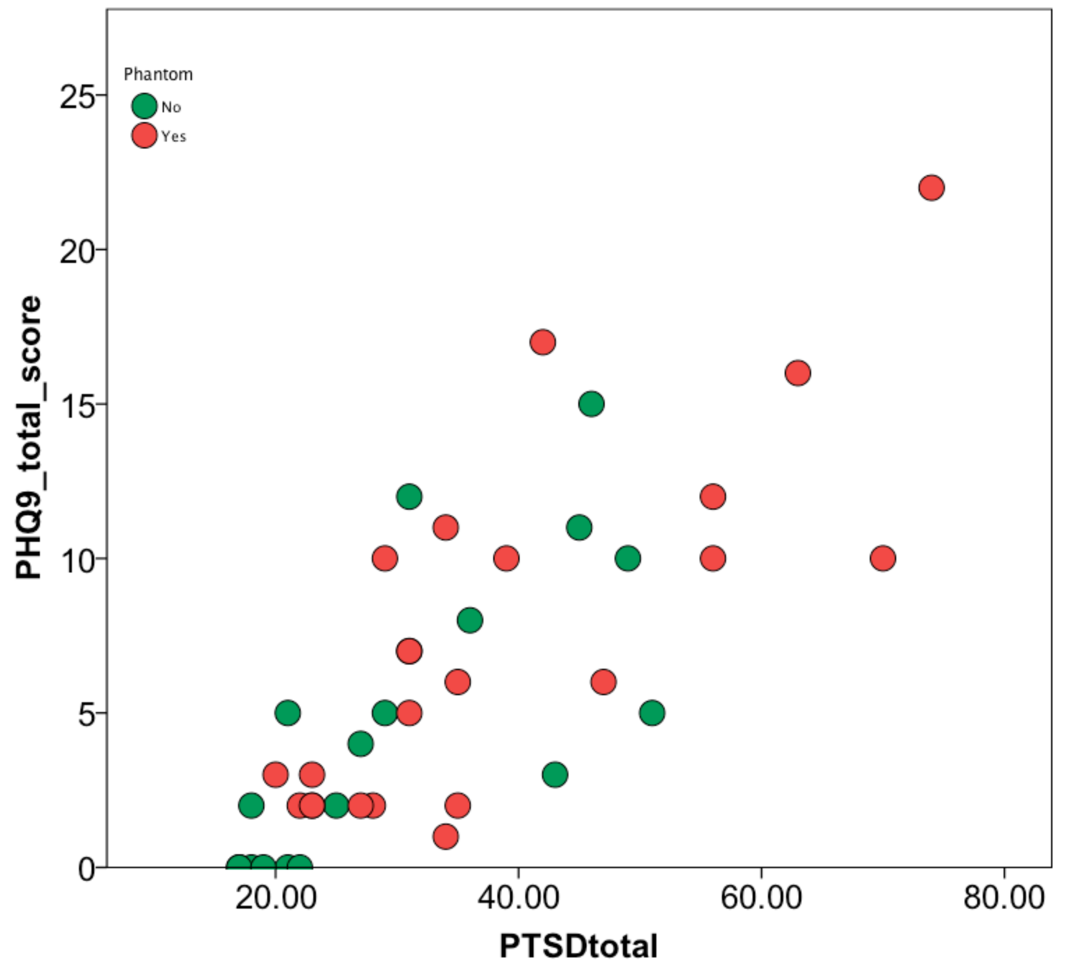
- We collect pain type and severity data using the following instruments:
- BPI, S-LANSS, PHQ 9, PCS, VAS, PTSD-M

Control = S LANSS <3
Case = S LANSS >2

Total N = 41	Controls (mean)	Cases (mean)	P value
Regional anesthesia catheter used (%)	31.2	16	NS
Ever smoker (%)	81.2	32	<0.05
Age	25	26	NS
BMI	25.7	26.6	NS
S-LANSS total	10	14	<0.05
S-LANSS indicator	0.88	4.48	<0.001
VAS (0-100)	4	24	0.001
PTSD total	28.9	37.8	<0.05
BPI worst	1	4	<0.05
BPI interfere	0.5	2.3	<0.05
PCS total	3	10	0.01
Stump pain (%)	0	56	<0.001
Phantom pain (%)	0	56	<0.001

Results

- There is a clear relationship between perceived impact of pain on quality of life and severity of PTSD symptoms
- This is independent of whether the pain is residual limb type or phantom



Comment

- This study, and the newly funded follow on intervention study, will provide detailed data regarding the epidemiology and molecular characteristics of the different subtypes of post amputation pain in military service personnel.

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1. Inclusion/Exclusion Criteria

VIPER ID ______________________________

Inclusion Criteria - A "No" answer to either point below excludes the patient from this study.

1. Patient is military health care beneficiary age 18 years or older, undergoing treatment at WRNMMC with the diagnosis of post injury amputation of all or part of one limb.	☐ Yes ☐ No
2. Amputation injury occurred more than 3 months but less than 18 months prior to enrollment.	☐ Yes ☐ No

Exclusion Criteria - A "Yes" answer to any of the points listed below excludes the patient from this study.

1. Severe Traumatic Brain Injury - Primary major head trauma and diagnosis of traumatic brain injury resulting in documented, permanent or prolonged cognitive deficits that would preclude participation in the study, i.e. decreased intellectual capacity, marked memory deficits or inability to communicate verbally or in writing.	☐ Yes ☐ No
2. Significant cognitive deficits that would preclude participation in the study.	☐ Yes ☐ No
3. Substantial hearing loss without alternative means of communication.	☐ Yes ☐ No
4. Documented spinal cord injury with permanent or persistent deficits.	☐ Yes ☐ No
6. Evidence of ongoing tissue damage pain, infection, bone spur or poorly fitting prosthesis.	☐ Yes ☐ No
7. Hip disarticulation	☐ Yes ☐ No

2. Demographics

Informed Consent

Is there a signed consent form in the shadow chart?
☐ Yes
☐ No

Subject Details

Gender
☐ Female
☐ Male

Ethnicity
☐ Hispanic or Latino
☐ NOT Hispanic or Latino
☐ Unknown / Not Reported

Race (check all that apply)
☐ American Indian/Alaska Native
☐ Asian
☐ Native Hawaiian or Other Pacific Islander
☐ Black or African American
☐ White

Age (years) ______________________________

Height (cm) ______________________________

Weight (kg) ______________________________

BMI ______________________________

Post Amputation BMI ______________________________

Do you currently smoke or have you ever smoked in the past?
☐ Yes
☐ No

What year did you start smoking? ______________________________

How many pack(s) per day did/do you smoke? ______________________________

If you have stopped smoking, what year did you stop? ______________________________

3. Past Medical History

Has a medical doctor ever diagnosed you with any of these symptoms? Check all that apply.

- ☐ Myocardial infarct (+1)
- ☐ Congestive heart failure (+1)
- ☐ Peripheral vascular disease (+1)
- ☐ Cerebrovascular disease (except hemiplegia) (+1)
- ☐ Dementia (+1)
- ☐ Chronic pulmonary disease (+1)
- ☐ Connective tissue disease (+1)
- ☐ Ulcer disease (+1)
- ☐ Mild liver disease (+1)
- ☐ Diabetes (without complications) (+1)
- ☐ Diabetes with end organ damage (+2)
- ☐ Hemiplegia (+2)
- ☐ Moderate or severe renal disease (+2)
- ☐ Solid tumor (non metastatic) (+2)
- ☐ Leukemia (+2)
- ☐ Lymphoma, Multiple myeloma (+2)
- ☐ Moderate or severe liver disease (+3)
- ☐ Metastatic solid tumor (+6)
- ☐ AIDS (+6)
- ☐ Heterotrophic ossification

Total points: ______________________________

4. Amputation & Injury

If you have more than one amputation, please refer to the one which causes you the most pain in answering these questions.

Site of amputation
- ☐ Left leg
- ☐ Right leg
- ☐ Left arm
- ☐ Right arm

High or low amputation site (eg above knee or elbow)
- ☐ High
- ☐ Low

Was a regional anesthesia catheter placed?
- ☐ Yes
- ☐ No
- ☐ Unknown

When? (yyyy) ______________________________

How many days was infusion maintained? ______________________________

Time since amputation procedure (months) ______________________________

Injury mechanism
- ☐ IED / blast injury
- ☐ Gunshot wound
- ☐ Motor vehicle accident
- ☐ Crush Injury
- ☐ Other

JTTR score ______________________________

5. Brief Pain Inventory (Short Form)

Please rate your pain by entering the one number (between 0 and 10) that best describes your pain at its WORST in the last 24 hours. ________________________________

Please rate your pain by entering the one number (between 0 and 10) that best describes your pain at its LEAST in the last 24 hours. ________________________________

Please rate your pain by entering the one number (between 0 and 10) that best describes your pain on the AVERAGE. ________________________________

Please rate your pain by entering the one number (between 0 and 10) that tells how much pain you have RIGHT NOW. ________________________________

In the last 24 hours, how much relief have pain treatments or medications provided? Please enter the one percentage that most shows how much RELIEF you have received.

- ☐ 0% - No Relief
- ☐ 10%
- ☐ 20%
- ☐ 30%
- ☐ 40%
- ☐ 50%
- ☐ 60%
- ☐ 70%
- ☐ 80%
- ☐ 90%
- ☐ 100% - Complete Relief

For the remaining questions, please enter the one number that describes how, during the past 24 hours, pain has interfered with your daily living. "0"=DOES NOT INTERFERE; "10" = COMPLETELY INTERFERES

a) General Activity ________________________________

b) Mood ________________________________

c) Walking Ability ________________________________

d) Normal Work (includes both work outside the home and housework) ________________________________

e) Relations with other people ________________________________

f) Sleep ________________________________

g) Enjoyment of Life ________________________________

6. S-LANSS Pain Score

This questionnaire can tell us about the type of pain that you may be experiencing. Please note where you feel your pain. If you have pain in more than one area, explain the one main area where your worst pain is. If you have multiple amputations, please focus on the one that gives you the most pain.

Please indicate how bad your pain (that you have shown on the diagram) has been in the last week where: '0' = no pain; '10' = pain as severe as it could be ________________________________

Following are 7 questions about your pain noted in the diagram. Think about how your pain that you showed in the diagram has felt OVER THE LAST WEEK. Please choose the descriptions that best match your pain. These descriptions may, or may not, match your pain no matter how severe it feels. Only answer the questions that describe your pain.

1. In the area where you have pain, do you also have 'pins and needles', tingling or prickling sensations?	☐ NO - I don't get these sensations. (0) ☐ YES - I get these sensations often. (5)
2. Does the painful area change colour (perhaps looks mottled or more red) when the pain is particularly bad?	☐ NO - The pain does not affect the colour of my skin. (0) ☐ YES - I have noticed that the pain does make my skin look different from normal. (5)
3. Does your pain make the affected skin abnormally sensitive to touch? Getting unpleasant sensations or pain when lightly stroking the skin might describe this.	☐ NO - The pain does not make my skin in that area abnormally sensitive to touch. (0) ☐ YES - My skin in that area is particularly sensitive to touch. (3)
4. Does your pain come on suddenly and in bursts for no apparent reason when you are completely still? Words like 'electric shocks', jumping and bursting might describe this.	☐ NO - My pain doesn't really feel like this. (0) ☐ YES - I get these sensations often. (2)
5. In the area where you have pain, does your skin feel unusually hot like a burning pain?	☐ NO - I don't have burning pain. (0) ☐ YES - I get burning pain often. (1)
6. Gently rub the painful area with your index finger and then rub a non-painful area (for example, an area of skin further away or on the opposite side from the painful area). How does this rubbing feel in the painful area?	☐ The painful area feels no different from the non-painful area. (0) ☐ I feel discomfort (pins/needles, tingling/burning) in the painful area. (5)
7. Gently press on the painful area with your finger tip then gently press in the same way onto a non-painful area (the same non-painful area that you chose in the last question). How does this feel in the painful area?	☐ The painful area does not feel different from the non-painful area. (0) ☐ I feel numbness or tenderness in the painful area. (3)

Total: (added by RN) ________________________________

7. PTSD Checklist

Below is a list of problems and complaints that veterans sometimes have in response to a stressful military experience. Please read each one carefully and choose the appropriate answer.

Repeated, disturbing memories, thoughts or images of a stressful military experience?

☐ Not at all
☐ A little bit
☐ Moderately
☐ Quite a bit
☐ Extremely

Repeated, disturbing dreams of a stressful military experience?

☐ Not at all
☐ A little bit
☐ Moderately
☐ Quite a bit
☐ Extremely

Suddenly acting or feeling as if a stressful military experience were happening again (as if you were reliving it)?

☐ Not at all
☐ A little bit
☐ Moderately
☐ Quite a bit
☐ Extremely

Feeling very upset when something reminded you of a stressful military experience?

☐ Not at all
☐ A little bit
☐ Moderately
☐ Quite a bit
☐ Extremely

Having physical reactions (e.g., heart pounding, trouble breathing or sweating) when something reminded you of a stressful military experience?

☐ Not at all
☐ A little bit
☐ Moderately
☐ Quite a bit
☐ Extremely

Avoid thinking about or talking about a stressful military experience or avoid having feelings related to it?

☐ Not at all
☐ A little bit
☐ Moderately
☐ Quite a bit
☐ Extremely

Avoid activities or talking about a stressful military experience or avoid having feelings related to it?

☐ Not at all
☐ A little bit
☐ Moderately
☐ Quite a bit
☐ Extremely

Trouble remembering important parts of a stressful military experience?

☐ Not at all
☐ A little bit
☐ Moderately
☐ Quite a bit
☐ Extremely

Loss of interest in things that you used to enjoy?

☐ Not at all
☐ A little bit
☐ Moderately
☐ Quite a bit
☐ Extremely

Feeling distant or cut off from other people?
- ☐ Not at all
- ☐ A little bit
- ☐ Moderately
- ☐ Quite a bit
- ☐ Extremely

Feeling emotionally numb or being unable to have loving feelings for those close to you?
- ☐ Not at all
- ☐ A little bit
- ☐ Moderately
- ☐ Quite a bit
- ☐ Extremely

Feeling as if your future will somehow be cut short?
- ☐ Not at all
- ☐ A little bit
- ☐ Moderately
- ☐ Quite a bit
- ☐ Extremely

Trouble falling or staying asleep?
- ☐ Not at all
- ☐ A little bit
- ☐ Moderately
- ☐ Quite a bit
- ☐ Extremely

Feeling irritable or having angry outbursts?
- ☐ Not at all
- ☐ A little bit
- ☐ Moderately
- ☐ Quite a bit
- ☐ Extremely

Having difficulty concentrating?
- ☐ Not at all
- ☐ A little bit
- ☐ Moderately
- ☐ Quite a bit
- ☐ Extremely

Being "super alert" or watchful on guard?
- ☐ Not at all
- ☐ A little bit
- ☐ Moderately
- ☐ Quite a bit
- ☐ Extremely

Feeling jumpy or easily startled?
- ☐ Not at all
- ☐ A little bit
- ☐ Moderately
- ☐ Quite a bit
- ☐ Extremely

Has anyone indicated that you've changed since the stressful military experience?
- ☐ Yes
- ☐ No

8. Patient Health Questionaire-9

Over the last 2 weeks, how often have you been bothered by any of the following problems?

1. Little interest or pleasure in doing things
- ☐ Not at all - 0
- ☐ Several days - 1
- ☐ More than half the days - 2
- ☐ Nearly every day - 3

2. Feeling down, depressed or hopeless
- ☐ Not at all - 0
- ☐ Several days - 1
- ☐ More than half the days - 2
- ☐ Nearly every day - 3

3. Trouble falling or staying asleep, or sleeping too much
- ☐ Not at all - 0
- ☐ Several days - 1
- ☐ More than half the days - 2
- ☐ Nearly every day - 3

4. Feeling tired or having little energy
- ☐ Not at all - 0
- ☐ Several days - 1
- ☐ More than half the days - 2
- ☐ Nearly every day - 3

5. Poor appetite or overeating
- ☐ Not at all - 0
- ☐ Several days - 1
- ☐ More than half the days - 2
- ☐ Nearly every day - 3

6. Feeling bad about yourself - or that you are a failure or have let yourself or your family down.
- ☐ Not at all - 0
- ☐ Several days - 1
- ☐ More than half the days - 2
- ☐ Nearly every day - 3

7. Trouble concentrating on things, such as reading the newspaper or watching television.
- ☐ Not at all - 0
- ☐ Several days - 1
- ☐ More than half the days - 2
- ☐ Nearly every day - 3

8. Moving or speaking so slowly that other people could have noticed. Or the opposite - being so fidgety or restless that you have been moving around a lot more than usual.
- ☐ Not at all - 0
- ☐ Several days - 1
- ☐ More than half the days - 2
- ☐ Nearly every day - 3

10. If you checked off any problems, how difficult have these problems made it for you to do your work, take care of things at home or get along with other people?
- ☐ Not difficult at all
- ☐ Somewhat difficult
- ☐ Very difficult
- ☐ Extremely difficult

Total (Entered by RN) ________________________________

9. PCS

We are interested in the types of thoughts and feelings that you have when you are in pain. Listed below are thirteen statements describing different thoughts and feelings that may be associated with pain. Please indicate the degree to which you have these thoughts and feelings when you are experiencing pain.

1. I worry all the time about whether the pain will end.
   - ☐ 0 = Not at all
   - ☐ 1 = To a slight degree
   - ☐ 2 = To a moderate degree
   - ☐ 3 = To a great degree
   - ☐ 4 = All the time

2. I feel I can't go on.
   - ☐ 0 = Not at all
   - ☐ 1 = To a slight degree
   - ☐ 2 = To a moderate degree
   - ☐ 3 = To a great degree
   - ☐ 4 = All the time

3. It's terrible and I think it's never going to get any better.
   - ☐ 0 = Not at all
   - ☐ 1 = To a slight degree
   - ☐ 2 = To a moderate degree
   - ☐ 3 = To a great degree
   - ☐ 4 = All the time

4. It's awful and I feel that it overwhelms me.
   - ☐ 0 = Not at all
   - ☐ 1 = To a slight degree
   - ☐ 2 = To a moderate degree
   - ☐ 3 = To a great degree
   - ☐ 4 = All the time

5. I feel I can't stand it anymore.
   - ☐ 0 = Not at all
   - ☐ 1 = To a slight degree
   - ☐ 2 = To a moderate degree
   - ☐ 3 = To a great degree
   - ☐ 4 = All the time

6. I become afraid that the pain will get worse.
   - ☐ 0 = Not at all
   - ☐ 1 = To a slight degree
   - ☐ 2 = To a moderate degree
   - ☐ 3 = To a great degree
   - ☐ 4 = All the time

7. I keep thinking of other painful events.
   - ☐ 0 = Not at all
   - ☐ 1 = To a slight degree
   - ☐ 2 = To a moderate degree
   - ☐ 3 = To a great degree
   - ☐ 4 = All the time

8. I anxiously want the pain to go away.
   - ☐ 0 = Not at all
   - ☐ 1 = To a slight degree
   - ☐ 2 = To a moderate degree
   - ☐ 3 = To a great degree
   - ☐ 4 = All the time

9. I can't seem to keep it out of my mind.
   - ☐ 0 = Not at all
   - ☐ 1 = To a slight degree
   - ☐ 2 = To a moderate degree
   - ☐ 3 = To a great degree
   - ☐ 4 = All the time

10. I keep thinking about how much it hurts.

- ☐ 0 = Not at all
- ☐ 1 = To a slight degree
- ☐ 2 = To a moderate degree
- ☐ 3 = To a great degree
- ☐ 4 = All the time

11. I keep thinking about how badly I want the pain to stop.

- ☐ 0 = Not at all
- ☐ 1 = To a slight degree
- ☐ 2 = To a moderate degree
- ☐ 3 = To a great degree
- ☐ 4 = All the time

12. There's nothing I can do to reduce the intensity of the pain.

- ☐ 0 = Not at all
- ☐ 1 = To a slight degree
- ☐ 2 = To a moderate degree
- ☐ 3 = To a great degree
- ☐ 4 = All the time

13. I wonder whether something serious may happen

- ☐ 0 = Not at all
- ☐ 1 = To a slight degree
- ☐ 2 = To a moderate degree
- ☐ 3 = To a great degree
- ☐ 4 = All the time

Total (entered by RN) ______________________________

10. Phantom Limb Pain

If you have more than one amputation, please focus on the one that causes you the most pain. __________________________________

Do you experience non-painful feelings in any part of the amputated arm and/or leg?

- ☐ Yes
- ☐ No

How often do you experience these feelings?

- ☐ A few times a year
- ☐ A few times a month
- ☐ A few times a week
- ☐ A few times a day
- ☐ A few times an hour
- ☐ Always

Do you experience pain in any part of the amputated arm and/or leg?

- ☐ Yes
- ☐ No

If so, how often?

- ☐ A few times a year
- ☐ A few times a month
- ☐ A few times a week
- ☐ A few times a day
- ☐ A few times an hour
- ☐ Always

11. Prosthesis

If you have more than one amputation, please answer these questions as they relate to the amputation that gives you the most pain.

Do you currently use a prosthesis?	☐ Yes ☐ No
Did you have stump pain prior to the use of a prosthesis?	☐ Yes ☐ No
Does wearing the prosthesis worsen your pain?	☐ Yes ☐ No
If you have a prosthesis, how often do you use it?	☐ Daily, 8 hours or more ☐ Daily, 4-8 hours ☐ Daily, less than 4 hours ☐ Not daily ☐ Never

12. Residual Limb Questions

If you have more than one amputation, please refer to the one which causes you the most pain in answering these questions.

Do you have any pain in the stump?
- ☐ Yes
- ☐ No

Does stump pain prevent you from using your prosthesis?
- ☐ Yes
- ☐ No

If you experience stump pain, how often does it occur?
- ☐ Never
- ☐ A few times a year
- ☐ A few times a month
- ☐ A few times a week
- ☐ A few times a day
- ☐ A few times an hour
- ☐ Always

13. Complex Regional Pain Syndrome Questions

If you have more than one amputation, please refer to the one which causes you the most pain in answering the questions.

Is your amputated arm/leg more sensitive than the other side? (If your other side is also injured, please use your arm as comparison).	☐ YES ☐ NO
Did the sensitivity change after amputation?	☐ YES ☐ NO
Is your amputated arm/leg WARMER than the other side? (If your other side is also injured, please use your arm as comparison).	☐ YES ☐ NO
Did the temperature change after amputation?	☐ YES ☐ NO
Is your amputated arm/leg COLDER than the other side? (If your other side is also injured, please use your arm as comparison).	☐ YES ☐ NO
Did the temperature change after amputation?	☐ YES ☐ NO
Is your amputated arm/leg a different color than the other side? (If your other side is also injured, please use your arm as comparison).	☐ YES ☐ NO
Did the color change after amputation?	☐ YES ☐ NO
Does your amputated arm/leg sweat more or less than the other side? (If your other side is also injured, please use your arm as comparison).	☐ YES ☐ NO
Did the sweating change after amputation?	☐ YES ☐ NO
Does your amputated arm/leg swell more than the other side? (If your other side is also injured, please use your arm as comparison).	☐ YES ☐ NO
Did the swelling change after amputation?	☐ YES ☐ NO
Does the amputated arm/leg cramp more than the other side? (If your other side is also injured, please use your arm as comparison).	☐ YES ☐ NO
Did the cramping change after amputation?	☐ YES ☐ NO

14. Neuroma/Focal Neuralgia Pain Questions

If you have more than one amputation, please refer to the one which causes you the most pain in answering the questions.

Does pain cover your whole stump or only part of it?
- ☐ Whole
- ☐ Part

Can you touch a spot on your stump that triggers stump or phantom pain?
- ☐ YES
- ☐ NO

15. Exam and Visual Documentation

(As observed by examiner on this date)

Sweating Asymmetry	☐ Yes ☐ No
If yes, please specify:	☐ Increased on affected side ☐ Decreased on affected side
Color Asymmetry	☐ Yes ☐ No
If yes, specify: Affected side is	☐ Red ☐ Blue or pale ☐ Mottled ☐ Scar
Dystrophic Changes	☐ Yes ☐ No
If yes, please specify:	☐ Hair ☐ Skin
Motor Abnormalities	☐ Yes ☐ No
If yes, please specify:	☐ Tremor ☐ Dystonia
Is stump/residual limb pain present?	☐ Yes ☐ No
Allodynia to cotton ball test inside the painful area	☐ Yes ☐ No
Allodynia to cotton ball test outside the painful area	☐ Yes ☐ No
Is the pain localized to a discreet area?	☐ Yes ☐ No ☐ Unclear
Is there evidence of a neuroma or a positive Tinel's sign?	☐ Yes ☐ No

Record of Camera Image ID Numbers

Control Digital Image (DC_nnnn)	__________________________
Control Thermal Image (IR_nnnn)	__________________________
Stump Digital Image (DC_nnnn)	__________________________
Stump Thermal Image (IR_nnnn)	__________________________

16. Narcotic Medications (current/within last 24 hours)

Oral morphine (total daily dose in milligrams) __________________________________

Oral hydromorphone (total daily dose in milligrams) __________________________________

Oral hydro - morph equiv __________________________________

Oral oxycodone (total daily dose in milligrams) __________________________________

Oral oxyco - morph equiv __________________________________

Oral hydrocodone (total daily dose in milligrams) __________________________________

Oral hydro - morph equiv __________________________________

Oral oxymorphone (total daily dose in milligrams) __________________________________

Oral oxymorphone - morph equiv __________________________________

Oral codeine (total daily dose in milligrams) __________________________________

Oral codeine - morph equiv __________________________________

Oral methadone (total daily dose in milligrams) __________________________________

Oral methadone - morph equiv __________________________________

Transdermal fentanyl (patch strength in micrograms) __________________________________

Transderm fentanyl - morph equiv __________________________________

Parenteral morphine (total daily dose in milligrams) __________________________________

Parenteral morphine - morph equiv __________________________________

Parenteral hydromorphone (total daily dose in milligrams) __________________________________

Parenteral hydromorph - morph equiv __________________________________

Parenteral fentanyl (total daily dose in micrograms) __________________________________

Parenteral fentanyl - morph equiv __________________________________

17. Blood Samples

Time blood was drawn ______________________

How many hours since last meal? ______________________

Collected whole blood in one EDTA tube?
- ☐ No
- ☐ Yes

Collected whole blood in one BD P100 tube?
- ☐ No
- ☐ Yes

Collected whole blood in two PAXgene RNA tubes?
- ☐ No
- ☐ Yes

Collected whole blood in one PAXgene DNA tube?
- ☐ No
- ☐ Yes

18. Completion Data

Study Completion Information

Has patient completed study?	☐ No ☐ Yes
Did patient withdraw from study?	☐ Yes ☐ No
Reason patient withdrew from study	☐ Non-compliance ☐ Did not wish to continue in study ☐ Hospitalization ☐ Other
General Comments	__________________________________

Prevention of Chronic Pain After Surgical Nerve Injury: Amputation and Thoracotomy

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KEYWORDS

• Chronic pain • Surgical nerve injury • Amputation
• Thoracotomy • Neuropathic pain

ACUTE POSTSURGICAL PAIN

A surgical incision produces tissue damage, subsequent inflammation, and acute postoperative pain. Although most patients heal without long-term sequelae, procedures, such as amputation, thoracotomy, hernia surgery, coronary artery bypass, and mastectomy, impose a significant burden of persistent postsurgical pain.[1–3] However, amputation and thoracotomy represent two of the higher-risk procedures. These surgeries involve obligatory neurologic injury, often leading to a cascade of postinjury sensitization and chronic neuropathic pain.[1,4]

Although amputation and thoracotomy have different indications and are performed using different techniques, they demonstrate a remarkable similarity both in the severity of acute postoperative pain and in the incidence of persistent postsurgical neuralgic pain.[1] Our ability to control incisional and inflammatory pain in the immediate postoperative period has improved with the combined use of local anesthetics, opioids, and other systemic medications. However, our tools to avoid central sensitization following nerve injury remain limited.

In recent years, an increased emphasis has been placed on the prevention and management of postinjury chronic pain states secondary to the military conflicts in the Middle East and around the globe. Between 2001 and 2010, more than 1600 US military personnel underwent amputation following military trauma.[5] In addition, natural disasters, such as the 2010 Haitian earthquake, have created more than

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6000 amputees.[6] Amputation surgery for medical and vascular disease also remains common, with a national rate of approximately 188 lower extremity amputations per 100,000 people.[7] Given the combination of soft tissue, bone, and neurologic injury that occurs in the course of an amputation, initial management is often problematic; patients experience not only nociceptive pain but also acute neuralgia and occasionally the immediate onset of phantom limb pain.[8]

Similarly, thoracotomy is characterized by a high incidence of both severe acute pain and intractable postoperative pain.[9] Poor analgesia following thoracotomy leads to poor chest wall mechanics, impaired cough, and subsequent respiratory and infectious complications. Given the preexisting tenuous pulmonary function of many thoracotomy patients, further decreases in pulmonary function may lead to significant additional morbidity.[10,11]

An ideal perioperative analgesic regimen for surgeries, such as amputation and thoracotomy, would not only facilitate the immediate relief of suffering but would also reduce the burden of chronic postsurgical pain. Indeed, these goals seem physiologically linked given the correlation between the severity of perioperative pain and the prevalence of chronic pain.[12–14] Despite these observational associations, the prevention of chronic postsurgical pain has been more difficult to accomplish than initially proposed.[15–17] In this review, the authors discuss perioperative pain management techniques and modifiable risk factors to prevent chronic pain following amputation and thoracotomy.

CHRONIC POSTSURGICAL PAIN: AMPUTATION

Patients undergoing amputation experience a high level of both phantom and residual limb pain following surgery. Of these 2 complications, phantom limb pain has been more frequently discussed in the literature, with an estimated prevalence of 51% to 85%.[18–21] Residual limb pain is also reported after amputation, with a frequency of 45% to 74%.[22–24] Although residual limb pain phenomena, such as causalgia[25,26] and neuroma,[22,27] have been reported, they have not been systematically studied as separate entities in the residual limb.[23,27–30] Nonetheless, distinction between the residual limb pain subtypes of neuroma, complex regional pain syndrome, and somatic pain is important for research and clinical care because all postamputation pain subtypes may not equally respond to a given therapy.

The appropriate treatment and prevention of postamputation pain is also of functional significance for patients. In a study of 2694 patients with amputations, 51% had phantom limb pain severe enough to impair lifestyle more than 6 days per month and 27% experienced pain more than 15 hours per day.[20,31] The effects of residual limb pain may have even greater functional implications for the patients with amputations because of its impact on prosthetic use, ambulation, and rehabilitation.[23,32]

In 1984, it was reported that fewer than 10% of patients with phantom limb pain obtained prolonged pain relief from medical treatments,[31] and only limited progress has been made since that time.[22,33] Surgical techniques, including dorsal root entry zone lesions, surgical sympathectomies, and spinal cord stimulation, have also been used.[34–36] Currently, however, there is a lack of evidence to support the efficacy of these techniques.[37] There are promising data regarding improvements in phantom limb pain from body reimaging techniques with mirror box therapy; unfortunately, this intervention does not improve residual limb pain.[38]

CHRONIC POSTSURGICAL PAIN: THORACOTOMY

Persistent post-thoracotomy pain is described as “pain along the incision site that persists or recurs after thoracotomy for at least two months following the surgical

procedure."[4] The cause of chronic pain following thoracotomy is undoubtedly similar to that following amputation. Neurologic injury at the time of surgery is likely the source of neuropathic pain, central sensitization, and persistent postsurgical pain in these patients.[4]

Up to 60% of thoracotomy patients report intractable pain a month after surgery and 30% to 50% report pain at 1 to 2 years.[10,39,40] Many of these patients describe significant physical limitations and sleep disturbances months and even years after surgery.[41] Similar to amputation pain, there is a strong correlation between severe perioperative pain and the incidence of chronic post-thoracotomy pain.[42–46]

RISK FACTORS FOR DEVELOPING CHRONIC POSTSURGICAL PAIN

Although all patients who undergo amputation and thoracotomy experience peripheral nerve injury, not all develop persistent neuropathic pain. Therefore, predisposing risk factors must also be present for chronic postsurgical pain to develop. Regarding amputation, identified chronic pain risk factors include severe perioperative pain, psychosocial comorbidity, and genetic predisposition. In particular, the association between severe preoperative pain[12,14,47–49] and postoperative pain[13,46,50] and the development of chronic pain supports the critical importance of acute symptom management. Indeed, both pharmacologic evidence[51] and radiologic demonstration[52–54] suggests central nervous system reorganization and sensitization in patients with amputations. Logically, if the preoperative stimulus is removed, thereby reducing the pain memory, the risk of persistent pain following amputation may decrease. A similar correlation between severe perioperative pain and chronic pain is also well documented in patients undergoing thoracotomy.[42–46] These observed associations between acute symptoms and chronic pain were part of the theoretical foundation behind the preemptive use of regional anesthesia before amputation and thoracotomy.[15,48,55]

Psychosocial factors also have an impact on the risk of chronic postoperative pain. Comorbidities, such as preoperative anxiety[56,57] and depression,[22,47,58,59] correlate strongly with persistent postsurgical pain. A comprehensive preoperative evaluation to identify these risk factors may have an impact on reducing the burden of chronic postsurgical pain.[60]

Gender and genetic risk factors are also increasingly appreciated as important to the development of chronic pain following surgery.[61] Several gene single nucleotide polymorphisms that may contribute to the development of neuropathic pain have been identified. Detailed discussions of these genetic factors may be found in previous publications[62,63] but are outside the scope of this review.

Given our current ability to identify predisposing factors for developing chronic postsurgical pain, we can now risk stratify patients who need more intensive multimodal therapy.[64] In subsequent sections, the authors focus on analgesic interventions that have been studied to reduce the incidence of persistent postsurgical pain.

ACUTE PAIN MANAGEMENT TECHNIQUES

Although there are evidence-based guidelines for acute pain management following thoracotomy,[65] there are no established guidelines for symptom management following amputation because of the inconsistent outcomes and methodological limitations of studies to date.[66] Surgical techniques, such as traction neurectomy and nerve implantation into muscle, may lessen the incidence of symptomatic neuromas.[67] However, these changes in technique have not significantly decreased the prevalence of chronic postamputation pain.[22] Likewise, minimally invasive

thoracic surgery has not dramatically improved the incidence of moderate to severe pain following thoracotomy.[68]

Many of the techniques studied in recent years for managing postamputation and post-thoracotomy pain have been initiated preoperatively.[69] This preemptive effect is designed to reduce nociceptive traffic to the spinal cord and central nervous system. In animal models, painful neuropathy can be attenuated with local anesthetic pretreatment[70,71] or by aggressive early treatment of pain.[14] Preemptive and perioperative therapies have been studied in an effort to reduce the burden of both acute and chronic postsurgical pain.

EPIDURAL ANALGESIA: AMPUTATION

Epidural analgesia is a common modality used to control acute pain at the time of amputation. Given the association between severe preoperative pain and chronic pain, investigators have hypothesized that aggressive perioperative pain control with epidural catheter infusion will also lessen the incidence of chronic postamputation pain. In a 1988 unblinded study of preemptive epidural analgesia, 25 patients in the epidural group reported dramatically reduced phantom limb pain at both 6 and 12 months when compared with controls.[15] Similarly, in a 1994 case-controlled study, Jahangiri and colleagues[72] observed only an 8% incidence of phantom limb pain in 24 patients treated with epidural bupivacaine, clonidine, and diamorphine compared with a 73% incidence in the control group treated with systemic opioids.

Unfortunately, these early successes were not repeated in later studies subjected to greater methodological rigor. In a 1997 prospective study, Nikolajsen and colleagues[17] randomized patients to receive preoperative and postoperative epidural blockade or standard postoperative epidural analgesia. At 12 months, both groups had a significant incidence of phantom limb pain: 75% in the preoperative and postoperative block group and 69% in the standard epidural group. Although a nonepidural treatment group was not included in this study, the incidence of phantom limb pain in these 2 study arms was similar to the background prevalence of phantom limb pain noted in other studies.[21,24,73] In a follow-up article, Nikolajsen and colleagues[74] examined the effect of preoperative and intraoperative epidural analgesia on stump sensitization after amputation. Again, they found no significant improvements. These findings are consistent with other clinical studies demonstrating that the timing of an analgesic intervention is not of critical importance.[69]

The current de-emphasis of the preemptive analgesia paradigm, however, has not lessened the significance placed on effective pain relief at the time of surgery. Indeed, the importance of successful analgesia is further supported by the 2011 publication by Karanikolas and colleagues[75] assessing epidural versus systemic analgesia in 65 patients undergoing amputation. Nearly all patients receiving epidural infusion or effective systemic analgesia saw a reduction in the prevalence of phantom limb pain at 6 months when compared with the controls treated with nurse-delivered intramuscular opioids. This article supports the concept that the success of analgesia may be more important than the specific technique used.

EPIDURAL ANALGESIA: THORACOTOMY

Similar to the interventions used for amputation surgery, epidural infusion has also been the gold standard for pain relief following thoracic surgery.[76] Thoracic epidural analgesia provides superior postoperative pain control when compared with parenteral opioids[77,78] and also facilitates early extubation, rehabilitation, and decreased perioperative complications.[79] The Procedure Specific Postoperative

Pain Management working group (www.postoppain.org) recommends thoracic epidural or paravertebral blocks for thoracic surgery as the first-line approach.

Despite the documented efficacy of thoracic epidural analgesia in the perioperative setting, the technique still fails in a significant number of patients.[80] The reason for this is unclear, and multiple hypotheses include catheter malposition, opioid tolerance, or poor drug spread to nerves located on the operative side.[81–84] Currently, there is limited evidence to support the notion that epidural analgesia reduces the incidence of chronic post-thoracotomy pain.

REGIONAL ANALGESIA: AMPUTATION

As an alternative to epidural analgesia, several trials of perineural catheters have been conducted in an effort to improve both acute and chronic pain symptoms following amputation. Initial studies of surgically placed perineural catheters were encouraging. In 1991, Malawer and colleagues[85] reported excellent perioperative analgesia with nerve sheath catheters in patients with amputations, and Fisher and Meller[16] described the complete absence of phantom limb pain in 11 patients treated with this technique.

Additional trials of this technique, however, did not reproduce these initial positive results. In 1994, Elizaga and colleagues[86] observed no significant improvement in acute or chronic pain in patients treated with surgically placed catheters. Other studies have reported either modest[87] or no improvement in the incidence of phantom limb pain.[88] It is also notable that surgically placed perineural catheters seem to provide inferior acute analgesia when compared with other regional anesthesia and epidural techniques.[89] The inadequate perioperative analgesia may be secondary to the distal placement of the catheter with minimal blunting of sensation at the surgical site. It is unknown whether the reduction in acute analgesia from surgical catheters has implications for longer-term postsurgical pain.

Although the previously mentioned studies of surgically placed perineural catheters provided equivocal results for managing postamputation pain, other percutaneous catheter insertion techniques are now commonly used by anesthesiologists and provide some potential advantages.[90] First of all, catheters may be placed preoperatively and used in a preemptive fashion. Secondly, and more importantly, the catheters may be placed in a location proximal to the incision, improving postoperative analgesia.

Previous studies gave sporadic reports of effective management of amputation pain using proximal perineural catheters.[91–93] More recently, Borghi and colleagues[94] evaluated this technique in a more systematic manner and found that prolonged perineural catheter use provided effective acute analgesia and long-term reduction of phantom limb pain. Notable aspects of this study were the lack of preoperative infusion and the prolonged duration of postoperative catheter use (median catheter duration of 30 days). Although not a randomized trial, the investigators did find only a 16% incidence of phantom limb pain at 12 months follow-up. These results have not yet been duplicated but are quite encouraging.

REGIONAL ANALGESIA: THORACOTOMY

Similar to perineural catheter infusions for amputation pain, paravertebral nerve blockade also involves the delivery of local anesthetic to nerves after they exit the spinal canal. Single-injection techniques at multiple dermatomes and continuous paravertebral catheters are generally used to manage pain from thoracotomy surgery. The classic method uses a loss-of-resistance technique; however, nerve stimulator

localization[95] and ultrasound techniques are also well described.[96–98] Ultrasound guidance improves accuracy of paravertebral catheter placement and minimizes the risk of pleural puncture.[99,100] Karmarkar and Richardson[101,102] provide additional details about these techniques.

Recent studies suggest that paravertebral nerve block provides comparable analgesia to epidural infusion with greater hemodynamic stability[103] and a better short-term side-effect profile.[104] The side effects associated with thoracic paravertebral blockade are generally low, although local anesthetic toxicity, block failure, bleeding, and pleural puncture may occur.[101,105,106] It is thought that pulmonary function is preserved with paravertebral block, subsequently decreasing pulmonary morbidity.[3,107–109] Thus, paravertebral blockade along with epidural infusion is still recommended.

SYSTEMIC MULTIMODAL ANALGESIA

Despite the recent emphasis placed on the perioperative use of epidural analgesia and peripheral nerve blockade, these techniques alone may not be sufficient for the prevention of chronic postsurgical pain. Circulating humoral inflammatory factors also induce central sensitization and neuropathic pain,[110,111] providing scientific justification for using multimodal systemic analgesia. Multimodal strategies use concurrent therapies in an effort to maximize pain relief and minimize side effects, particularly those related to opioid analgesics.[112] Although opioid analgesics remain an important part of the acute pain protocol for amputation and thoracic surgery, their singular use is often not sufficient to provide effective systemic analgesia. In this review, the authors discuss adjuvant analgesics and novel nonopioid pain control strategies.

Nonsteroidal Antiinflammatory Drugs

Nonsteroidal antiinflammatory drugs (NSAIDs) have been extensively investigated in the perioperative period, and their use improves analgesia, reduces opioid requirements, and reduces opioid-related side effects.[113] Additionally, the question of preemptive analgesia from preoperative NSAID administration has been investigated in more than 20 trials. However, preoperative dosing improved symptom management in only 2 of these trials when compared with intraoperative and postoperative dosing, indicating that there is likely little or no preemptive effect from these drugs.[69]

Cyclooxygenase-2 (COX-2) inhibitors are sometimes preferred in the perioperative period given their decreased effect on platelet function. Similar to other NSAIDs, the COX-2 inhibitor celecoxib demonstrates improvement in acute analgesia with an opioid-sparing effect but no significant preemptive analgesic effect.[114–116] Celecoxib has demonstrated efficacy as part of a multimodal strategy for thoracic surgery.[117] Studies related to NSAID efficacy following amputation are lacking, but these analgesics should be considered given their documented effectiveness for acute pain. However, there is no current evidence that NSAID use prevents either chronic postamputation or post-thoracotomy pain.

Acetaminophen

Oral acetaminophen has enjoyed long-term use for managing acute pain, and intravenous (IV) acetaminophen has recently been approved in the United States. Although both forms have been used in the perioperative period, the IV formulation may have some advantages given its reliable pharmacokinetics and ease of administration.[118,119]

Because acetaminophen improves acute analgesia in patients undergoing thoracotomy, it is increasingly being used in the perioperative period, except in patients with significant liver disease.[120] Although there are concerns about the safety of chronic acetaminophen use, acute administration of up to 4 g/d seems to be safe in most patients.[121] Similar to NSAIDs, however, no studies have demonstrated that acetaminophen reduces chronic postamputation or post-thoracotomy pain. Nonetheless, given its minimal effect on platelet aggregation, perioperative bleeding, and renal function,[122] acetaminophen should be strongly considered in the perioperative setting.

Gabapentinoids: Gabapentin/Pregabalin

There has been significant interest in the use of gabapentinoids for neuropathic pain since their 1993 release in the United States. Because these drugs can inhibit Ca^{2+} currents and reduce neurotransmitter release associated with neural sensitization,[123] they have demonstrated efficacy in multiple neuropathic pain conditions.[124,125]

Gabapentin and pregabalin have been studied as a preemptive measure before surgery with evidence of decreased acute pain, opioid consumption, and improvement in opioid-related side effects.[126–128] Additionally, gabapentin is effective in reducing the severity of chronic phantom limb pain.[33] Despite the demonstrated efficacy of gabapentinoids in acute and chronic neuropathic pain, they have not been shown to prevent chronic phantom limb pain when given in the immediate postoperative period.[129] Although their use following amputation may be appropriate given their beneficial effect on acute postoperative pain, future research is needed to establish optimal timing, dosing, and efficacy of perioperative gabapentenoids.[128,130,131]

Clonidine

Clonidine, an $\alpha 2$ adrenergic agonist, plays a potential role in the treatment of neuropathic pain because of the expression of $\alpha 2A$ receptors at the site of nerve injury[132] as well as on local infiltrating macrophages and lymphocytes.[133] Clonidine administration decreases the local expression of inflammatory cytokines, such as TNF-α and IL-1β, and improves hypersensitivity following nerve injury.[134] Epidural and perineural clonidine have also been studied as a therapy for neuropathic pain[134] and have been used clinically in the treatment of chronic postamputation pain.[135,136] Because $\alpha 2A$-adrenoceptors and inflammatory cytokines play important roles in the production of postamputation chronic pain, clonidine deserves further investigation. It is generally well tolerated, but its clinical use is occasionally limited by dose-dependent side effects, such as hypotension and sedation.[137]

Ketamine

Ketamine is an antagonist of the N-methyl D-aspartate receptor known to be involved in central sensitization and neuropathic pain.[138] It has been used in the treatment and prevention of chronic pain following nerve injury, although randomized controlled efficacy trials are still lacking.[139] Ketamine has been investigated as a systemic drug[51,140] and an epidural drug[141] for amputation surgery and it has been shown to reduce stump sensitivity in the immediate postoperative period.[141] Although ketamine has also been found to reduce acute hyperalgesia and allodynia when given at the time of thoracic surgery,[142] it is not effective for treating chronic postamputation pain[141] or post-thoracotomy pain.[143]

SUMMARY AND FUTURE DIRECTIONS

Growing evidence suggests that multimodal analgesia, using a combination of catheter-based techniques[94,144] and systemic analgesics,[112,145,146] reduces the risk of chronic postsurgical pain. Comprehensive therapy is particularly important for patients undergoing high-risk surgeries, such as amputation and thoracotomy. With the recent demonstration that effective acute pain management, regardless of the method used, decreases the prevalence of phantom limb pain at 6 months,[75] we now have the scientific justification and the ethical obligation to treat these patients with the multiple tools at our disposal. Furthermore, because prolonged perineural catheter infusions may reduce the burden of postamputation pain,[94] we must reevaluate the postoperative treatment period. Therefore, rather than several days of recovery, we may need to consider prolonged therapies during the time of neurologic plasticity. If we can alter this postoperative remodeling process, we will have an additional tool to reduce the incidence of chronic postsurgical pain.

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ACUTE & PERIOPERATIVE PAIN SECTION

Original Research Articles

Epigenetics and the Transition from Acute to Chronic Pain

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Abstract

Objective. The objective of this study was to review the epigenetic modifications involved in the transition from acute to chronic pain and to identify potential targets for the development of novel, individualized pain therapeutics.

Background. Epigenetics is the study of heritable modifications in gene expression and phenotype that do not require a change in genetic sequence to manifest their effects. Environmental toxins, medications, diet, and psychological stresses can alter epigenetic processes such as DNA methylation, histone acetylation, and RNA interference. As epigenetic modifications potentially play an important role in inflammatory cytokine metabolism, steroid responsiveness, and opioid sensitivity, they are likely key factors in the development of chronic pain. Although our knowledge of the human genetic code and disease-associated polymorphisms has grown significantly in the past decade, we have not yet been able to elucidate the mechanisms that lead to the development of persistent pain after nerve injury or surgery.

Design. This is a focused literature review of epigenetic science and its relationship to chronic pain.

Results. Significant laboratory and clinical data support the notion that epigenetic modifications are affected by the environment and lead to differential gene expression. Similar to mechanisms involved in the development of cancer, neurodegenerative disease, and inflammatory disorders, the literature endorses an important potential role for epigenetics in chronic pain.

Conclusions. Epigenetic analysis may identify mechanisms critical to the development of chronic pain after injury, and may provide new pathways and target mechanisms for future drug development and individualized medicine.

Key Words. Epigenetics; Pain; DNA Methylation; Histone Deacetylase Inhibitors; RNA Interference

Introduction

In recent years, we have developed a better understanding of the cellular mechanisms that link inflammation, peripheral sensitization, and pain [1]. In addition, we have learned more about the human genetic code [2] and mutations (particularly single nucleotide polymorphisms [SNPs] and copy number variations) that are associated with specific chronic pain syndromes [3,4]. These physiologic and genetic advances, however, do not fully explain why one patient develops chronic pain following an injury, and another patient does not. Despite recent improvements in techniques for acute pain management, 30–50% of patients still develop chronic pain following surgeries such as amputation, thoracotomy, hernia repair, and mastectomy [5].

It is also notable that monozygotic twins may exhibit significantly different inflammatory and chronic pain phenotypes [6–8], indicating that the etiological basis of these

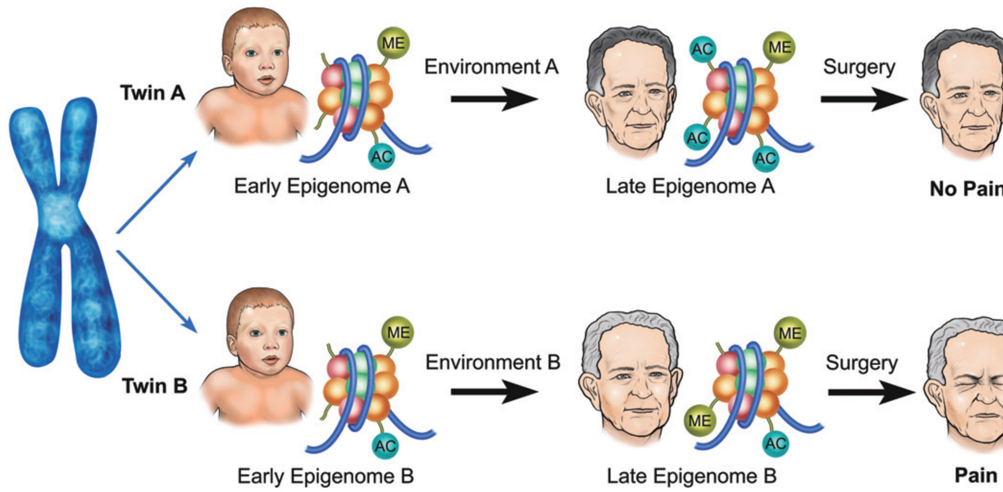

Figure 1 Epigenome and chronic pain. Twin A and Twin B demonstrate similar "epigenomes" at birth with few (if any) differences in methylation and acetylation patterns. Environmental factors throughout development affect histone acetylation patterns and cytosine methylation patterns, resulting in phenotypic differences by adulthood. With surgery or nerve injury, these epigenetic differences may result in differing risks of chronic pain.

disorders is not due simply to differences in genetic sequence. We now appreciate that response to injury is determined by complex interactions between the genome and the environment. These alterations might well be epigenetic in nature, i.e., heritable modifications that are not intrinsic to the genetic code, but that affect gene expression in a tissue-specific manner, resulting in an observable phenotype (Figure 1) [9].

Epigenetic processes are responsible for cellular differentiation during embryogenesis and are critical for normal development [10]. These processes also play an important role in memory formation, as correlations between hippocampal activity, DNA methylation, and histone phosphorylation in the brain have been found [11,12]. The spinal cord sensitization seen in painful conditions shares common mechanisms with the neural plasticity of memory formation [13], and it is likely that similar epigenetic mechanisms regulate both of these neural processes.

Multiple examples of the importance of epigenetic influences in development are found throughout nature. One of the best-described cases of environmental influence on gene expression involves the control of bee development by ingesting royal jelly. This nutritive substance induces changes in juvenile bee DNA methylation patterns and leads to development of the bee's phenotype to become a queen rather than a worker [14]. The concepts of epigenetic heritability and stability have also been described in plants [15] and mammals [16]. For instance, high-fat diets fed to paternal rats induce functional changes in β-islet cells of female offspring [16]. Similar modifications in DNA methylation were noted in the fathers and offspring, suggesting the nongenetic heritability of this metabolic disorder.

Nondevelopmental epigenetic modifications are also triggered by environment, nutrition, and stress [17–19], and may play a role in the onset of chronic pain following nerve injury [20,21]. We have long appreciated the importance of the psychosocial environment to the incidence and severity of chronic pain [22–27], and mounting evidence suggests that epigenetic mechanisms supply the link between disease expression and environment [18,28]. Nongenetic factors are important in the development of cancer [29,30], neurologic disorders [31], and painful disorders such as bladder pain syndromes [7], myofascial pain [32], and temporomandibular joint pain [8]. Twin disease models of neurodegenerative conditions [33], inflammatory periodontal disease [34], and autoimmune disease [35] demonstrate variable disease expression depending on the DNA methylation pattern [6].

Environmental factors alter gene expression and phenotype for painful disorders by inducing epigenetic modifications such as histone acetylation, DNA methylation, and RNA interference (RNAi) [36–38]. Following injury, expression of transcription factors such as nuclear factor-kappa B (NF-κB) is increased [39], sodium channels in the injured axon are upregulated [40], μ-opioid receptors in the dorsal root ganglion are downregulated [41,42], substance P expression is altered [43], and the dorsal horn of the spinal cord is structurally reorganized through axonal sprouting [44]. As with DNA variation, epigenetic modifications may be inherited and may be propagated over multiple cell divisions; however, they are flexible enough to respond to

modifying influences. This concept may in part explain how we interact with our environment at the (epi)genomic level, and is potentially of great importance in understanding the relationship between gene expression and complex diseases such as chronic pain.

Genetics, Epigenetics, and Pain

Over the past several decades, much has been written about the association of genetic polymorphisms and the development of chronic pain [45,46]. It was believed that, through knowledge of genetic variation, we could develop the foundation for individualized medicine that optimizes therapy for each patient based on one's specific genetic sequence [47]. Expectations for personalized medicine were high after completion of the human genome project [2], but thus far, our ability to use the genetic code to prevent or improve chronic pain has been somewhat limited [48]. It is the heretofore unquantifiable environmental effect that has been one of the limitations of genetic studies [45].

Multiple candidate gene association studies have been used for the investigation of pain, but have been limited by their focus on genomic regions where the pathophysiology is thought to be reasonably well understood. They are not designed to analyze painful conditions that result from interactions of multiple genes [49]. A few candidate gene polymorphisms have been linked to pain susceptibility, including catechol-O-methyltranferase (COMT). This gene modulates nociceptive and inflammatory pain and has been linked to temporomandibular joint pain syndromes [50]. Even studies of COMT, however, have demonstrated inconsistencies. Some investigators have found an association between a COMT SNP val158met [4,50] with increasing pain responses, while others failed to replicate these findings [51,52].

The SCN9A gene has also been studied as a marker for pain sensitivity. Mutations in this gene, which codes for the alpha-subunit of a voltage-gated sodium channel ($Na_v1.7$), are known to result in alterations of pain perception [53], and have been noted in rare pain disorders such as erythromelalgia and paroxysmal extreme pain disorder [54,55]. SCN9A polymorphisms have also been described in individuals who are insensitive to pain [3,56]. Although the implications of the SCN9A gene polymorphism are clear, clinical applications of this knowledge remain limited [47].

Genome-wide association studies (GWAS) have been used in an attempt to overcome some of the limitations of candidate gene analysis. These studies tell us where the genetic variation exists, but do not always fully explain the underlying biology. Furthermore, although GWAS have identified thousands of genetic variations in complex diseases, most of the variants confer only a modest risk with an odds ratio for disease of <1.5. These genetic variants, therefore, account for only a small fraction of the population attributable risk for heritable complex traits [57,58], implying a strong nongenetic predisposition to disease. GWAS directed toward painful conditions remain limited in number [45].

Specific Epigenetic Modifications

Histone Modifications

Histones octamers and their surrounding DNA form a nucleosome, the fundamental building block of chromatin (Figure 2A). The N-terminal histone tails may be modified by more than 100 different posttranslational processes including acetylation, phosphorylation, and methylation (Figure 2B). Most of the histone complex is inaccessible, but the N-terminal tail protrudes from the nucleosome and is therefore subject to additions that change the three-dimensional chromatin structure and subsequent gene expression [59,60]. One of the more common modifications involves acetylation. Histone acetyl transferases add acetyl groups, altering the histone protein structure. This change prevents the chromatin from becoming more compact, allowing transcription factors to bind more easily. This state of increased acetylation and "permissive chromatin" generally increases transcription activity and RNA production from that genetic sequence, especially when located in gene promoter regions [61,62]. Conversely, histone deacetylases (HDACs) remove acetyl groups from histones, generally suppressing gene expression. In concert, these activities serve important regulatory functions.

DNA Methylation

Another ubiquitous epigenetic modification involves methylation of DNA cytosine nucleotides. In this process, DNA methyltransferase enzymes (DNMT1, DNMT3A, and DNMT3B) add a methyl group to the 5-carbon of the cytosine pyrimidine ring, converting it to 5-methylcytosine. This methylation generally silences gene expression either by preventing the binding of transcription factors [63,64], or by attracting methylated DNA-binding proteins such as MeCP2 that themselves repress transcription (Figure 2C) [65,66]. The methylation process is vital for normal embryonic development and growth [67], and these methylation patterns are propagated during cell division.

The degree of cytosine methylation tends to mirror the degree of tissue specialization. For instance, DNA in neurologic tissue is highly methylated, while sperm DNA is relatively unmethylated [68]. More recent research has focused on the regulatory importance of cytosine methylation in promoter regions where methylation may silence a previously active gene sequence in the process of tissue specialization [69]. In addition to the cytosine nucleotides dispersed throughout the genome, there are regions particularly rich in cytosine-phosphate-guanine (CpG) linear sequences, described as "CpG islands" [70]. These "CpG islands" are found in promoter regions or first exons of approximately 60% of human genes, and are often unmethylated during development, allowing a transcriptionally active state [71]. Although promoter site methylation may

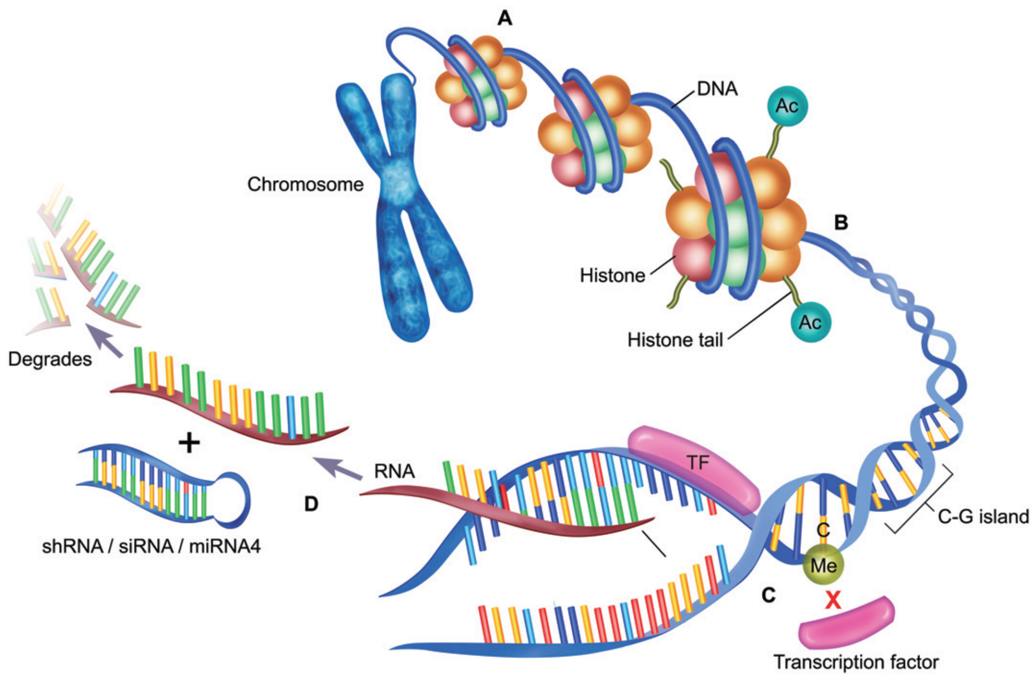

Figure 2 Epigenetic mechanisms. (A) DNA wraps around histone octamers to form a nucleosome, the fundamental building block of chromatin. (B) Histone proteins may be modified through several processes, including acetylation. The addition of an acetyl group to histone tails generally opens the chromatin structure and facilitates transcription factor binding, enhancing gene expression. (C) Methylation of cytosine nucleotides in C-G rich sequences ("CG islands") prevents the binding of transcription factors and generally silences gene expression. These CG islands are often found near promoter regions and serve a significant role in gene regulation. (D) Posttranscriptional regulatory mechanisms include short hairpin RNA (shRNA), small interfering RNA (siRNA), and micro RNA (miRNA) that bind RNA and induce their degradation.

silence gene expression during development, genes may still be reactivated even in specialized neurologic tissues [72,73]. This potentially modifiable plasticity of neural tissue methylation may hold promise for reversing the neurologic molecular remodeling that occurs during the transition from acute to chronic pain.

Several disease states, including cancer, schizophrenia, and opioid addiction, are associated with DNA methylation abnormalities [30,74–76]. In cancer, these altered methylation patterns may lead to tumor growth by downregulating tumor suppressor genes [30]. Methylated gene domains demonstrate not only stability, but also heritability [70]. The epigenetic influence across generations is demonstrated in rodent studies in which spermatogenesis is suppressed, and methylation patterns are altered for several generations after using the antiandrogenic compound vinclozolin during embryonic development [77].

Noncoding RNA

Gene expression can also be controlled by RNAi that involves endogenous molecules such as small interfering RNA (siRNA), microRNA (miRNA), and short hairpin RNA (shRNA). These small noncoding RNA molecules can silence gene expression by binding to mRNA and inducing subsequent degradation of the direct gene product (Figure 2D) [78]. These molecules can self-propagate through cell division and epigenetically transmit regulatory information across generations [79]. Interfering RNAs carry great therapeutic promise and have been used in animal trials for chronic neuropathic pain [80] and neurodegenerative disease [81], as well as in human clinical trials for cancer [82].

Our understanding of epigenetic processes has increased dramatically over the past decade. Efforts are currently underway, through such groups as the International Human Epigenome Consortium, to sequence and create maps of cell-specific DNA methylation and histone modifications [83].

Techniques of Epigenetic Analysis

There are many challenges in defining the specific epigenetic changes that lead to a particular disease state. Many earlier epigenomic studies have been limited by either inadequate genome survey or small sample size, and the

relationship in many diseases between phenotypic expression and epigenomic variation remains unclear [84]. It is unlikely that single gene epigenetic modification will explain the complex pain phenotypes seen after injury or surgery. Epigenome-wide association studies have been proposed as a possible solution to improve our understanding of the links between disease state and epigenetic modifications. Comprehensive epigenomic maps are currently being developed with promising future applications [84].

Another challenge with epigenetic studies and disease variation is the need for enhanced comprehension of the distinction between cause and consequence [84]. To fully understand if a particular biomarker represents the cause *of* a disease or the effect *from* a disease, we will need to perform analyses at multiple time points before and after the development of a disease. This initiative has already begun with the establishment of the U.S. National Institutes of Health Roadmap Epigenomics Mapping Consortium [85].

Regardless of the relationship between biomarkers and causation, however, epigenetic modifications throughout the course of a chronic disease can be used as biomarkers. In particular, DNA methylation is well suited as a potential predictive biomarker secondary to its relative chemical stability. Reliable biomarkers are critical if we are to develop personalized epigenetic interventions. Candidate markers would need to be found in an accessible space (blood), but still reflect the neurobiological process occurring at the proximal tissue (spinal cord/brain). Whether the circulating leukocyte epigenome can report on more inaccessible tissues (such as central nervous system [CNS]) is uncertain, but there is growing evidence that methylation patterns tend to be similar between proximal tissue and more easily accessible circulating blood cells. For example, it was recently shown that the pattern of CpG island methylation in the promoter region of the prodynorphin gene in both human brain tissue collected postmortem and matched peripheral blood mononuclear cells is virtually identical [86].

The burgeoning field of epigenetics is using novel technologies to measure these heritable, yet modifiable, patterns of transcriptional regulation. DNA methylation is analyzed through bisulfite sequencing that allows the epigenetic information present in the form of cytosine methylation to be retained during amplification (Figure 3B). Traditional molecular analysis of specific gene loci relies on the ability to amplify the DNA of interest using cloning and polymerase chain reaction (PCR) techniques. If this amplification is done, however, without somehow immortalizing the methylation status of a particular cytosine, that information will be lost after the first PCR cycle. To solve this problem, unmethylated cytosines can be modified through the bisulfite reaction, deaminating them to uracil. Methylated cytosines, however, are not deaminated by bisulfite, remaining unchanged during subsequent amplification. Probes can then be designed to determine whether a specific promoter region has retained a particular cytosine (previously methylated) or whether this cytosine has been converted to uracil (previously unmethylated). The methylation status of the promoter can then be determined using the cytosine/uracil ratio.

Histone protein modifications have also been studied since 1988 through a process of chromatin immunoprecipitation (ChIP) (Figure 3A) [87]. This process involves fragmentation of the chromatin and immunoprecipitation using an antibody to the protein or modification of interest. For example, an antibody to a specific acetylation site on histone H3 is used to precipitate all DNA associated with that particular acetylated histone. Following immunoprecipitation, the DNA fragments are then typically identified through microarray hybridization. More recently, “next generation sequencing” (NGS) technologies have been combined with ChIP, providing a high resolution, genome-wide analysis of histone modification. Whereas microarray techniques analyze regions of the genome previously identified, NGS carries the possibility of capturing all the DNA fragments isolated by immunoprecipitation [71]. These NGS technologies will continue to expand our understanding of epigenetic changes and the chromatin regulatory state throughout the genome.

The Role of Epigenetic Modification in the Transition from Acute to Chronic Pain

Prevention of chronic pain after injury has been the focus of numerous previous trials involving interventions such as multimodal analgesics and catheter-based local anesthetic infusions [88–90]. Although these techniques are successful in reducing the burden of acute pain [91], they have not succeeded in dramatically reducing the incidence of chronic post-injury or post-surgical pain [92–94]. The shortcomings of our preventive strategies are most pronounced following surgeries that have a higher risk for developing chronic pain such as amputation, thoracotomy, hernia repair, coronary artery bypass, and mastectomy [5,95,96].

Our therapeutic limitations may be partially due to our inability to prevent the epigenetic changes that occur following injury and surgery. A patient’s gene expression profile changes rapidly in the post-injury period [97], with over 1,000 genes activated in the dorsal root ganglion alone after nerve injury [98]. There is significant evidence for epigenetic control of this gene activation in the transition from acute to chronic pain. First, immunologic response and inflammatory cytokine expression are under epigenetic control [99,100]. Second, glucocorticoid receptor (GR) function, which affects pain sensitivity, inflammation, and the development of autoimmune disease, is modulated both through posttranslational mechanisms and DNA methylation [101–103]. Third, genes such as glutamic acid decarboxylase 65 that code for pain regulatory enzymes in the CNS are known to be hypoacetylated and downregulated in inflammatory and nerve injury pain states [104]. Finally, epigenetic modifications are involved in opioid receptor regulation and function, with implications for endogenous pain modulation systems and pain severity [63,76].

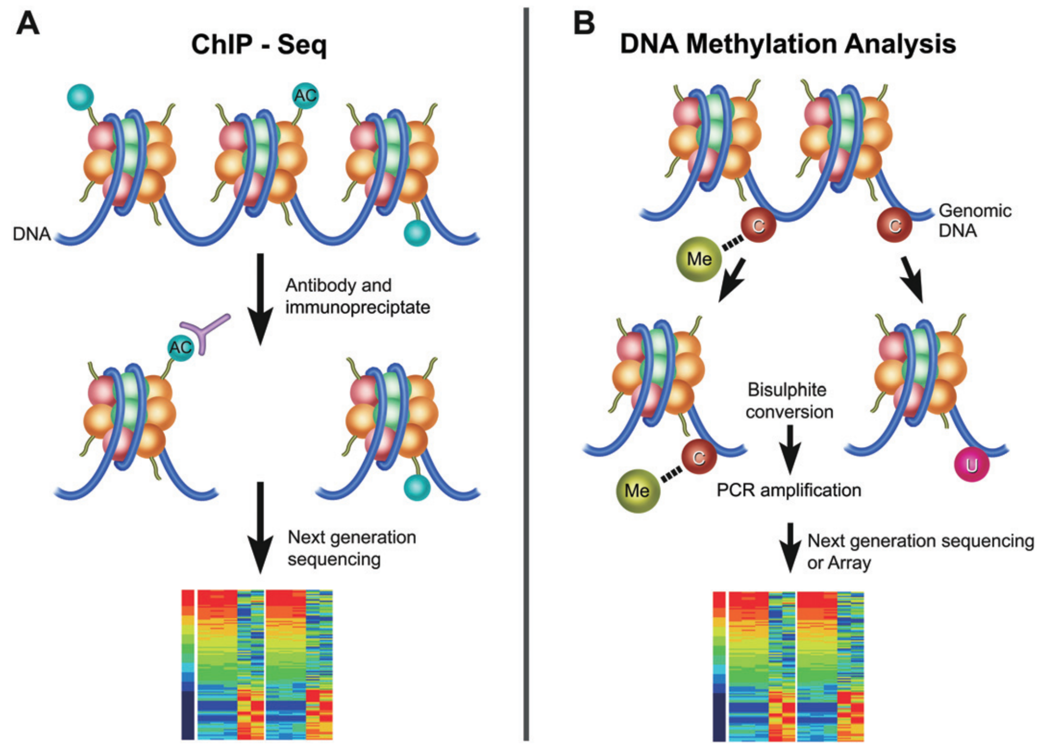

Figure 3 Laboratory techniques in epigenetics. (A) In ChIP-seq analysis, an antibody is used on chromatin to immunoprecipitate and select for acetylation and other histone modifications. The results may then be analyzed through several techniques including genome-wide next generation sequencing. In this manner, the histone acetylation patterns of a particular tissue may be determined. (B) The analysis of DNA methylation employs bisulfite sequencing to convert unmethylated cytosines to uracil. This process does not affect the methylated cytosines. The methylation patterns can be calculated by comparing the ratio of cytosine to uracil.

The important link between epigenetic regulation and pain is also supported by studies involving intervertebral disc degeneration and chronic low back pain. Tajerian et al. found that DNA methylation of an extracellular matrix protein, secreted protein, acidic, rich in cysteine, is linked to accelerated disc degeneration both in humans and in animal models of this disease [38]. The correlation between pain and epigenetics is additionally observed in a study of DNA methylation in human cancer where endothelin receptor type B (EDNRB) is heavily methylated and downregulated in painful squamous cell carcinoma (SCC) lesions [105]. The investigators noted similar findings in their mouse model of SCC, and were able to improve mechanical allodynia when EDNRB transcription was virally augmented [105]. These human and animal studies strongly support a role for gene methylation in regulating the pain experience.

Cytokines

Injury and autoimmune disease are characterized by excessive cytokine production, and anti-cytokine therapies have been successfully used to treat painful conditions such as ankylosing spondylitis [106,107] and neuropathy [108,109]. The link between cytokine expression and pain is supported by the demonstration of T-cell infiltration and inflammatory interleukin (IL) release in animal models of neuropathic pain [110]. Furthermore, interventions that modify the immune response to injury also reduce pain. Such modifications include depletion of mast cells [111], reduction of peripheral macrophages using clodronate [112], and impairment of complement activation and neutrophil chemotaxis [113].

One of the inflammatory master switches, nuclear factor-κB (NF-κB), induces multiple cytokines [114] and cyclooxygenase [115]. NF-κB is epigenetically regulated by acetylation and remodeling of chromatin [114,116,117]. When activated, this transcription factor demethylates and induces cytokines such as Tumor necrosis factor-alpha (TNF-α), IL-1, IL-2, and IL-6 [118,119]. Activation of NF-κB is associated with autoimmune and neurodegenerative disease [120]. Conversely, inhibition of NF-κB reduces pain behavior after peripheral nerve injury [121].

The link between epigenetically induced cytokine production and pain intensity has been noted in multiple disease models such as migraine headache [122], diabetes [114], and osteoarthritis [99]. In osteoarthritis, DNA demethylation at specific CpG sites in human chrondrocytes produces aberrant expression of inflammatory cytokines (IL-1β) and metalloproteinases [99]. Thus, cytokine-induced painful joint damage appears to be epigenetically modulated.

GRs

Glucocorticoids are important endogenous regulators that appear to protect against excessive inflammatory response following injury. Stress-induced glucocorticoid production suppresses immune cell release of IL-6, TNF-α, and other inflammatory cytokines [123]. Exogenous glucocorticoids also have potent anti-inflammatory actions and are used extensively in the treatment of autoimmune disease and painful conditions. However, not all patients respond equally to their clinical effects, and it is believed that glucocorticoid resistance is a likely mechanism in the development of autoimmune disease and chronic pain [124].

The GR is controlled by a system of complex regulatory mechanisms, and clinical response to glucocorticoids correlates with the number of intracellular GRs [125]. Normally, individuals demonstrate variable GR promoter methylation [103] and variable response to glucocorticoid therapy [126]. Diverse methylation patterns are believed to lead to the use of alternative promoter sites and subsequent alteration in GR sensitivity [103].

GR expression is also modified by maternal care, grooming, diet [127,128], and early-life stresses [129,130]. Human studies have demonstrated epigenetic alterations in GRs of patients who previously suffered abuse [131]. The style of maternal care appears to specifically affect methylation patterns of exon 1_7 of the GR promoter, epigenetically linking receptor function and early-life experience [132]. Abnormalities in GR-mediated immune cell function may lead to the development of inflammatory adult phenotypes [133] and autoimmune disorders such as rheumatoid arthritis [101,134]. GR dysfunction may also play a role in fatigue, chronic pain states, and fibromyalgia [102,135]. These maternally influenced expression patterns, however, are not necessarily permanent and have been reversed in cross-fostering parent studies [136]. The GR appears to provide a potential link between injury, environmental stresses, and the severity of chronic pain.

Opioid Receptors

Both demethylating agents and HDAC inhibitors increase expression of the μ-opioid receptor [137], indicating that the endogenous opioid system is under significant epigenetic control. Consistent with these laboratory findings, increased CpG methylation has been noted in the promoter regions of the μ-opioid receptors of heroin users, consistent with receptor downregulation [76], Likewise, DNA methylation of the proenkephalin gene promoter inhibits transcription and gene expression of this opioid peptide [63].

Beyond the direct role of methylation in the regulation of opioid peptide expression, spinal opioid receptor activity also appears to be partially modulated by central GRs [138]. This association is of particular importance given the synergy between the increased central expression of GR following peripheral nerve injury [139] and direct epigenetic manipulation of the endogenous opioid system [63,137]. The interaction between modifications of the GR and the opioid receptor demonstrates the complex role that epigenetic alterations play in controlling the inflammatory and pain-modulating pathways.

“Epigenetic Intervention” to Prevent Chronic Pain

Genetic studies have taught us that variability in pain sensitivity results from multiple genetic and environmental factors. Environmental influences upon pain severity have been previously described and linked to early-life stress [47,140–143]. Although precise mechanisms have yet to be elucidated, epigenetic modifications are increasingly appreciated as a likely factor in this linkage [36,104,122].

Our need for targeted therapies has never been greater. Multiple analgesic drugs are now in use; however, most of these share a common function with opioids or anti-inflammatory medications. These medications have improved symptoms in some patients, but have created the additional morbidities of systemic toxicity, opioid tolerance, and addiction. Our options for safe and effective treatments for chronic pain remain limited with few recent “breakthroughs.”

Since the sequencing of the human genome, there have been increasing calls for “personalized medicine” that tailors drug therapy to a patient’s pain phenotype [47,144]. Although such therapies have demonstrated some efficacy as cancer treatments [145–147], we have not yet had great success with targeted pain therapies. We will now review some of the potential targets for “personalized epigenetic intervention” (Table 1).

Intervention: HDAC Inhibition

Given the association between histone deacetylation and cancer, neurodegenerative disease, and pain, histone deacetylase inhibitors (HDACis) have been evaluated as therapeutic agents for these diseases [30,36,148]. Thus far, HDACis are primarily used in cancer therapy. In these patients, HDACis alter the balance of acetylation/deacetylation and activate genes that suppress tumor growth and invasion [30,149–152]. In neurodegenerative disease, HDACis have been evaluated secondary to their ability to induce neural growth and to improve memory [153]. HDACis have also demonstrated evidence for

Table 1 Epigenetically active drugs and their mechanisms

Epigenetics Mechanism	Drug	Action	Clinical Use	Comments
Histone deacetylase inhibitor	Valproic acid	Inhibits classes I and II HDAC	Seizures, pain	Effective for migraine prophylaxis
	Givinostat	Inhibits classes I and II HDAC	Juvenile idiopathic arthritis	Effective in human arthritis trial
	Tricostatin A (TSA)	Inhibits classes I and II HDAC	Laboratory only	Produces analgesia in animal models. Enhances μ-opioid receptor transcription
	Suberoylanilide hydroxamic acid (SAHA)	Inhibits classes I HDAC	Laboratory only	Produces analgesia in animal models
DNA methylation	Glucosamine	Prevents demethylation of IL-1β gene promoter	Arthritis pain	Common clinical use; effect on IL-1β reduces inflammatory cytokine production
	Valproic acid	Induces demethylation of reelin promoter	Seizures, pain	Reelin modulates NMDA function and pain processing
	L-methionine	Induces methylation at glucocorticoid receptor promoter gene	Dietary supplement	Alters experimental stress response; used as dietary supplement for arthritis
RNA interference	siRNA targeted to NMDA receptor subunits	Gene silencing of NR1 and NR2 subunits of NMDA	Experimental	Produces analgesia in animal models
	siRNA to P2X3	Gene silencing of P2X3	Experimental	Produces analgesia in animal models; no observed neurotoxicity with intrathecal use
	siRNA to TNF-α	Gene silencing of TNF-α	Experimental	Produces analgesia in animal models

analgesia in both inflammatory and neuropathic pain [151,154,155]. The clinical effect of many of these drugs is thought to be partially attributed to reduced production of inflammatory cytokines such as TNF-α and IL-1β [156].

HDACis are organized into several different structural groups. Trichostatin A (TSA) and suberoylanilide hydroxamic acid (SAHA) are hydroxamate-based HDACis. TSA inhibits both class 1 (ubiquitously expressed) and class 2 (selectively expressed) HDACs, whereas SAHA exhibits greater selectivity for class 1 HDAC. TSA produces analgesia in animal models with an associated decrease in expression of transient receptor potential type-1 cation channel (TRPV1) and protein kinase Cε [157]. SAHA reduces the nociceptive response of animals during the second phase of the formalin test [154]. These drugs increase acetylation of the transcription factor p65/Re1A, which enhances gene expression of the metabotrobic glutamate receptors (mGlu2) in dorsal root ganglia neurons. Activation of these mGlu2 receptors inhibits primary afferent neurotransmitter release in the dorsal horn of the spinal cord and provides analgesia in animal models of neuropathic pain [158]. TSA also enhances μ-opioid receptor transcription [159], indicating partial HDAC modulation of the endogenous opioid system.

Another HDACi, Givinostat, has not only demonstrated evidence of analgesia in animal models, but also efficacy in a human trial for juvenile idiopathic arthritis. Although randomized studies have not yet been performed, its use for this autoimmune inflammatory disease is especially encouraging given its relative lack of systemic toxicity [160].

The most commonly used HDACi, valproic acid (VPA), is part of the aliphatic-based drug class that inhibits classes I and II HDACs [151,161], and is effective following systemic or intrathecal administration [162,163]. VPA is of particular interest because it has been successful with long-term clinical use [164]. Although it is now used predominantly to treat chronic painful conditions [163–165], its inhibition of HDAC and potential to prevent specific epigenetic alterations may lead to preemptive use in the acute setting. It is not yet clear whether VPA-induced analgesia results from HDAC inhibition or its ability to potentiate gamma amino butyric acid (GABA) in the CNS.

Although therapies based on HDAC inhibition have been effective in treating pain and oncologic disease, nonspecific HDACis such as TSA affect the regulation of multiple

genes, which increases the possibility of side effects with this therapy [166,167]. The success of future drug development will likely depend upon our ability to target specific subclasses of HDACs that selectively alter pain processing without the toxicities of nonselective agents. The importance of this selectivity concept has been demonstrated in a mouse model in which a full knockout of the HDAC4 gene (a class IIa HDAC) is lethal, whereas a conditional knockout of this gene provides analgesia [168]. Further investigations of HDAC subclass function are needed in order to identify novel drug targets.

Intervention: DNA Methylation

DNA methylation is another key epigenetic mechanism. Methylation patterns, although generally stable throughout the genome, are responsive to pharmacologic intervention. One common medication that appears to act through epigenetic mechanisms is glucosamine [169]. In arthritis models, it has been demonstrated that glucosamine prevents demethylation of the IL-1β gene promoter, thereby decreasing expression of this cytokine. Decreased IL-1β subsequently reduces NF-κB expression and downstream inflammatory cytokine production [119,170].

In addition to its function as an HDAC inhibitor, VPA induces demethylation of multiple genes [171]. One of these important genes encodes for reelin, a glycoprotein synthesized by GABAergic neurons of the CNS [172,173]. Reelin modulates N-methyl-D-aspartate (NMDA) receptor function [174], and is important for sensory processing [175]. Mutations of this gene cause alterations in mechanical and thermal hypersensitivity [173], which indicates the potential significance of VPA regulation of reelin in the development of chronic pain.

L-methionine administration has also been tested as a potential drug for epigenetic intervention. This amino acid appears to increase methylation patterns of the GR gene, thereby altering the hypothalamic-pituitary-adrenal response to stress [176]. In addition, dietary methyl supplementation in an animal model improves the health and longevity of offspring [177]. Both of these findings suggest that nutritional status partially controls the activity of the GR and its role in inflammatory disease.

The combined action of pharmacologic DNA demethylation and HDAC inhibition increases activity at the proximal promoter site of the μ-opioid receptor gene, increasing μ-opioid receptor expression [137]. Carried out in concert, these processes may represent an important balance that allows less stable histone modifications to lead to more stable changes in DNA methylation, thus facilitating longer-term modifications in the endogenous opioid receptor system.

Intervention: RNAi

Epigenetic therapies based on RNAi also hold promise for preventing and treating chronic pain. These methods target specific disease pathways.

RNAi is an endogenous mechanism for gene silencing in plants [178] and mammals [179], and involves subgroups such as siRNA, miRNA, and shRNA. Given their ability to silence undesirable gene products in malignancy, these small RNA molecules have been used for cancer therapy [82]. They have also been shown to improve chronic neuropathic pain [80].

siRNA targeted for the NR2 subunit of NMDA receptors abolishes formalin-induced pain behavior in rats [180]. Likewise, injection of siRNA aimed at the NR1 subunit of the NMDA receptor alleviates experimentally induced allodynia in mice [181]. Successful RNAi studies have targeted TRPV1 channels [182], brain-derived neurotrophic factor [183], cytokines such as TNF-α [184], and pain-related cation channels ($P2X_3$) [80]. Importantly, direct intrathecal administration of siRNA targeting $P2X_3$ in animals has not demonstrated significant toxicity [80], indicating that this intervention may be applicable to humans in coming years.

Conclusions

The transition from acute to chronic pain is a complex process involving local inflammation and nociceptor activation that may resolve in some patients and may lead to the development of chronic pain in others. As we learn more about the various ways that injury and environment change gene expression, we can begin to elucidate disease mechanisms and gain insight into potential therapies. Epigenetic alterations such as DNA methylation, histone acetylation, and RNAi are necessary for normal tissue specialization and neurologic development. However, these same modifications play a significant role in the induction of the chronic pain phenotype following neurologic injury.

In contrast to the genetic determinism inherent in genomic studies, the field of epigenetics strives to understand the environmental control over gene expression. Such knowledge will open up opportunities for developing novel analgesics. Future personalized therapies will likely be based on epigenetic interventions that alter the transcriptional expression that occurs in chronic pain states. Given the strong mechanistic implications of epigenetic modifications in the development of chronic pain, and our current treatment limitations, we possess both the promise of epigenetic tools and the imperative to prevent the transition from acute to chronic pain.

Authors' Contribution

TB, TV, and AS conceived, wrote, and performed the final editing of this manuscript. Medical illustrations were created in collaboration with Stan Coffman from Medmedia Solutions, Durham, NC. We also wish to thank Kathy Gage, BS, Duke University Department of Anesthesiology, for her editorial assistance in the preparation of this work.

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RE: PT110575 - "Regional Anesthesia and Valproate Sodium for the Prevention of Chronic Postamputation Pain"

STATUS: RECOMMENDED FOR FUNDING

Dear Thomas Buchheit:

Congratulations! On behalf of the Department of Defense (DoD) office of the Congressionally Directed Medical Research Programs (CDMRP), I am pleased to inform you that the Fiscal Year 2011 (FY11) Psychological Health/Traumatic Brain Injury (PH/TBI) Research Program Applied Neurotrauma Research Award with Clinical Trial - Partnering Option application you submitted was recommended for funding. This decision was based on the scientific merit of your application and its relevance to the programmatic goals of the PH/TBI.

In order to start the award process, additional information is needed. Your timely submission of all applicable documents in the appropriate formats will expedite the award process and release of funds. A CDMRP Science Officer, who will be your scientific and technical point of contact throughout the life of this award, may contact you for further information regarding various aspects of your application. For example, your Science Officer will contact you about Animal Use and Human Use documents that may be needed for your project. Together, you will set a target date for the submission of your complete animal use or human use appendix documents.

A Grants/Contract Specialist from the US Army Medical Research Acquisition Activity will contact the Business Official (person authorized to conduct negotiations) at your institution to begin award negotiations. All official negotiations of the budget, terms, and conditions of any resulting award will be limited to the Business Official of your institution and the Government Grants/Contract Specialist.

Your performance on any previous CDMRP awards will be considered during negotiations. You are advised to review your current and past awards to ensure that all required information has been submitted, including all technical reporting and regulatory oversight documents, as this may impact the negotiation and award process.

If you are withdrawing your application, please co-sign a letter of withdrawal with an administrator from the Sponsored Programs Office at your institution and upload it under the "Required Award Information" tab on the CDMRP eReceipt website (https://cdmrp.org).

To expedite the award process, please answer the post-submission questions found under the "Required Award Information" tab on the CDMRP eReceipt website (https://cdmrp.org). You and your institution are responsible for ensuring that there is no duplication of the science, budget, or level of effort in separately funded studies in which you were or currently are involved. If you received funding for any portion of this application from another source, or if any portion of the proposed work has already started, please indicate so under this tab.

After providing the information requested under the "Required Award Information" tab, upload or provide the following, for all sites where the DoD-funded work will be conducted, under the "Required Award Documents" tab by July 27, 2012:

- Facility Safety Assurance documents (required only if the institution is not listed as approved at: https://mrmc.amedd.army.mil/assets/docs/sse/Facility_Safety_Plan_Approved_Institutions.pdf)
- Environmental Compliance documents
- PI Safety Assurance documents
- Updated details on all existing and pending support for yourself and key personnel, including the title of the project, goals, specific aims/tasks, estimated start date and end date, level of effort (percentage or calendar months) in the project, and point of contact at the funding agency. Provide a cover letter signed by a sponsored programs official, certifying that this information is current and accurate, and addresses any scientific or financial overlap.

The CDMRP staff and I look forward to working with you to realize the vision of the PH/TBI, and we encourage you to share the news of your success with your colleagues and the community. Please direct any questions to the CDMRP at help@cdmrp.org or 301-682-5507. A copy of this letter is being made available to the Sponsored Programs Office at your institution.

Sincerely,

Susan M. Dellinger
Grants Officer